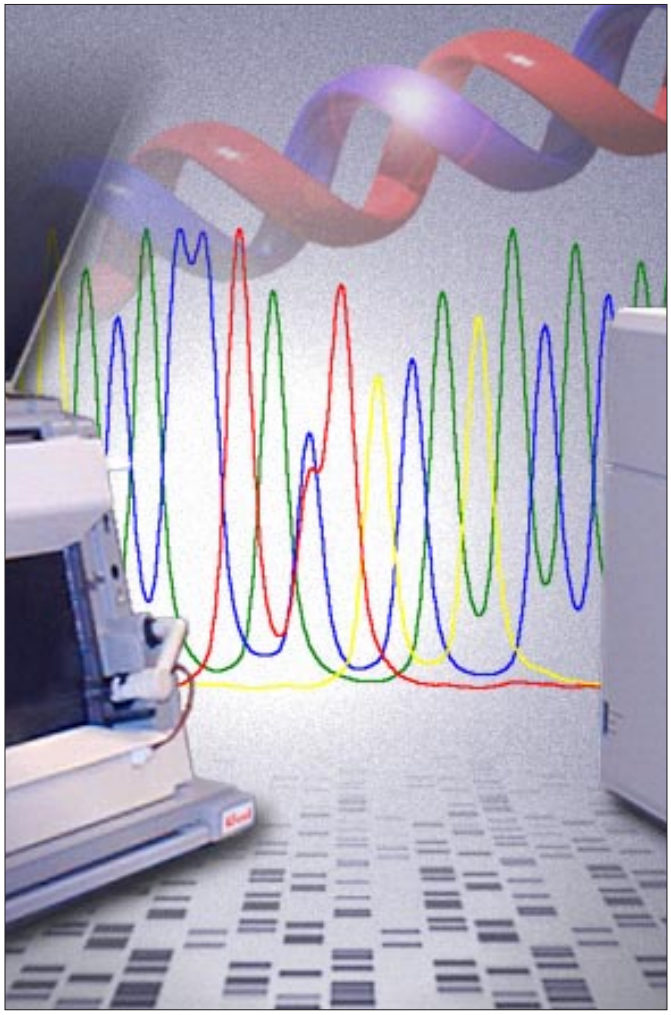
ALFwin™

Sequence Analyser 2.00



User Manual

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Edition AA

Amersham Biosciences

Important user information

Reading this entire manual is recommended for full understanding of the use of this product.

Should you have any comments on this manual, we will be pleased to receive them at:

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Preface

This User Manual describes the use and operation of ALFwin Sequence Analyser 2.00 for the control of sequencing runs on the ALF family instruments and analysis of the sequencing results.

Specific information contained in the various chapters of the User Manual can be accessed using the Table of Contents or Index. The contents of each chapter can be summarised as follows:

Contents

Installing the software	This instructs you how to install and uninstall ALFwin Sequence Analyser.
Chapter 1	This gives an introduction to the key features of ALFwin Sequence Analyser, some general tips in using the software, and an overview of the conventions used in the User Manual.
Chapter 2	This presents a step by step guide on how to make a first run.
Chapter 3	This introduces the ALFwin Administrator module and tells you how to create and edit user profiles.
Chapter 4	This introduces the ALFwin Control module and tells you how to perform a sequencing run including working with casebooks.
Chapter 5	This introduces ALFwin Sequence Analyser module and tells you how to process and present your data, and prepare the data for export to other applications.
Chapter 6	This introduces the Sequence Alignment application that resides in the ALFwin Sequence Analyser module.
Chapter 7	This presents possible solutions to some of the problems that you may encounter using ALFwin Sequence Analyser.

Appendix A	This details the computer hardware system requirements.
Appendix B	This is a short guide to using Windows 95.
Appendix C	This describes the base calling algorithm used to process raw data.
Appendix D	This lists the keyboard shortcuts associated with ALFwin Sequence Analyser.
Appendix E	Reference list.
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Installing the software

You should check that your computer complies with the minimum hardware specification requirements detailed in Appendix A and that you are using Microsoft Windows 95 operating system.

To install the software

1. Check that all analysis instruments are switched off.
2. Turn on the computer. Windows 95 should start automatically.
3. Insert Disc 1 into drive A.
4. Click on the Start button in the desktop taskbar and select **Run**.
5. Enter **a:\setup** at the command prompt. Click on **OK**.
6. Follow the instructions on the screen.
7. Remove the final diskette from drive A before performing the computer restart.

To uninstall the software

1. Locate and select **Settings:Control Panel** from the Start button menu.
2. Select **Add/Remove Programs**.
3. Select ALFwin Sequence Analyser 2.00 in the list and click on the **Add/Remove** button.
4. Follow the instructions on the screen.

Note: When you uninstall the program, only the program is removed. All the files and folders created by users will NOT be removed. If there are any shared files, you will be asked to retain or discard them. If you are unsure, retain the files and these will not interfere with the normal running of your computer.

1 Introduction

To obtain the best sequencing results using the ALF family instruments, Amersham Biosciences has developed a control and analysis sequencing software called ALFwin Sequence Analyser 2.00. ALFwin Sequence Analyser gives you total control over the sequencing run and collection of the run data. Data can then be processed to generate the actual DNA sequences for each of the clones and these generated sequences can be aligned for comparisons. Present your results on the screen in gel or curve view and print out a report of the analysis that you have performed. Finally, export the results for direct use or for further analysis in other software applications.

1.1 Key features of ALFwin Sequence Analyser

- Modularised software
  The software is modularised for convenient control, analysis and administration of sequencing runs and sequence data.
- Programmed casebooks
  Programmed casebooks contain all information for total control of each run. Include the settings for experiment run conditions, notes on samples and other experimental factors, post-run actions, data file handling and full documentation logging.
- Simultaneous control and analysis
  ALFwin Control allows simultaneous control of two ALF family instruments while also evaluating results from other runs.
- Real-time monitoring
  Sequencing runs are monitored in real-time on the screen.
- Intuitive interaction
  The graphical interface has been designed for intuitively interactive control and analysis. Work with the data in several modes including the Curve view and the Gel (fluorogram) view.
- Full data processing with accurate base calling
  Processing involves the automatic extraction of the sequence from the run data. The base calling algorithms are extremely accurate providing user confidence in the results, and advanced base calling functions can be programmed to accommodate different levels of heterozygocity. Raw data obtained from the sequencing run can be processed either as post run actions directly after the run or later within ALFwin Sequence Analyser.

- Sequence alignment
  The sequences can be aligned with one another for comparison of different sequences to locate similarities and differences between the samples.
- Compatible export formats
  DNA sequence export formats are ASCII, DNASIS, EMBL and GCG. DNA sequences with data curves included can be exported as STADEN SCF. Data curves can also be exported as pictures by saving as Windows MetaFiles, and using the copy clone and copy to clipboard functions. Aligned sequences can be exported in MSF and Phylip formats. Additionally, you can convert the data to TIFF files for direct use in other application software.
- Customised reports
  Customised reports can be created from the result file, the aligned sequences and also from the casebook.
- Windows 95
  The software has a modern user interface developed for Windows 95.
- Updates on the Web
  For the latest information, user methods, run conditions and more for ALFwin Sequence Analyser, contact the Amersham Biosciences internet home pages *www.amershambiosciences.com*.

1.2 Using ALFwin Sequence Analyser

1.2.1 Windows 95 operating system

ALFwin Sequence Analyser has been designed to use the features and benefits of the Windows 95 operating system to make your use of ALFwin Sequence Analyser as effective and intuitive as possible. It is assumed that you have a working knowledge of Microsoft Windows 95 and you are recommended to read appropriate operating system literature for further information. A basic summary of useful Windows 95 operations are presented in Appendix B.

1.2.2 Menu commands

Many of the menu commands in ALFwin Sequence Analyser can be activated using the toolbar buttons, keyboard shortcuts and the right mouse button menu. The availability of these command options is dependent on the active field or window in which you are currently working. The function of a toolbar button is displayed when you place the mouse pointer over a button. Keyboard shortcuts are summarised in Appendix D. Right mouse button menu commands are quickly found through use of the program.

1.2.3 Tip of the day

The Control and Sequence Analyser modules always start up with a **Tip of the Day** dialogue to give you useful tips on using the program. If you do not want to view this dialogue at start-up, deselect the **Show Tips at StartUp** option.

To view tips of the day, you can select the **Tip of the Day** command in the **Help** menu.

1.2.4 On-line Help

ALFwin Sequence Analyser is supplied with a context sensitive On-line help. If you want an explanation about a specific dialogue in which you are working, either click on the **Help** button in the dialogue or press the <F1> key on the keyboard.

You can also access the help topics by selecting the **Help Topics** command in the **Help** menu. This allows you to locate help topics based on the contents, index or keyword search.

1.3 Using this manual

This User Manual provides you with step-by-step instructions on how to specify the experimental conditions of a run, control a run, process raw data, align sequences, analyse the results and prepare the data for export into other applications.

1.3.1 Typographical conventions

Menu commands, the names of dialogue boxes and windows, the contents of dialogue boxes windows, and option buttons are written with a **bold helvetica** typeface. Menu commands are written in the order of the menu name and then the command, separated by a colon.

For example:

> "Select **File:Save As** to display the **Save As** dialogue. Locate the destination drive and directory and enter a file name. Click on **Save**."

This directs you to click on the **File** menu and select the command, **Save As**. A dialogue called **Save As** is displayed in which you must locate the destination directory for the saved file and give the file a name. You then click on the button called **Save** in the dialogue to execute the save command.

Some menu commands also have shortcut keys on the keyboard, which are written within < > marks.

2 Making your first run

The aim of this chapter is to take you through your first sequencing run, analyse the data and print a report. This will introduce you to the three ALFwin Sequence Analyser modules; Administrator, Control and Sequence Analyser. You will gain practical experience of using ALFwin Sequence Analyser with an instrument to obtain sequencing data. For the purposes of the first run, you should use the Ready-To-Go® ALFexpress M-13 Ladder (order no. 27-4560-80), which you can obtain from your local Amersham Biosciences representative.

Read through this chapter carefully before making a run.

Consult the appropriate instrument user manual for information about the instrument you are using.

2.1 Before starting a run

2.1.1 Safety

Before starting a run, it is essential that:

- you know how to prepare the instrument for a sequencing run. You should follow the relevant user manual for the ALF family analyser with which you are working.
- you are familiar with the safety information concerned with using the instrument and the use and disposal of hazardous chemicals.

Attention:
If you have any doubts or questions concerning aspects of safety, contact your local Amersham Biosciences representative.

2.1.2 Pre-run activities

Before you start your first run, the following must be ready:

- the gel cassette for the instrument must be assembled and the gel freshly cast and polymerised in the cassette
- sufficient running buffer (2 litres) must be prepared for the instrument
- the lower buffer reservoir must be located into the front of the instrument

- a pair of electrodes must be available
- the communication cable from the instrument must be connected to an available COM (serial) port on the computer.

2.2 Making a run

This procedure refers to the ALF*express* II instrument.

2.2.1 Turning on the instrument and computer

1. Turn on the computer. Windows 95 [1]should start automatically.

2. Turn on the ALF*express* II instrument. The green and red indicator lamps on the bottom left side at the front of the instrument should illuminate steadily.

3. Launch ALFwin Administrator by selecting **ALFwin Software** from the Windows 95 Start button menu options. The **ALFwin - Administrator** dialogue is shown.

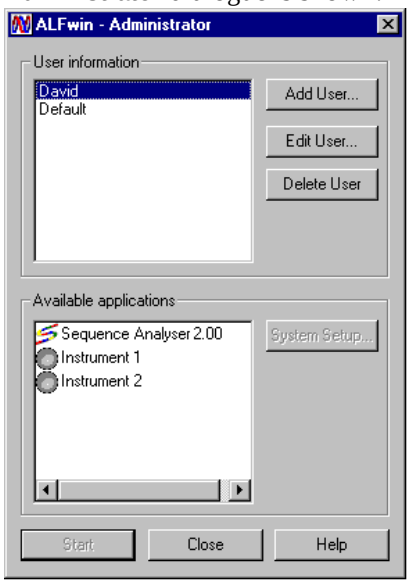

Figure 2-1. ALFwin - Administrator dialogue.

1. If you are unfamiliar with Windows 95, read Appendix B, A short guide to Windows 95, or consult the appropriate user manual for the Windows 95 operating environment.

2.2.2 Adding a user

You can enter details for each new user of ALFwin Sequence Analyser. To add a new user:

1. From the **ALFwin - Administrator** dialogue, click on **Add User**. The **Add or Edit User** dialogue is shown.

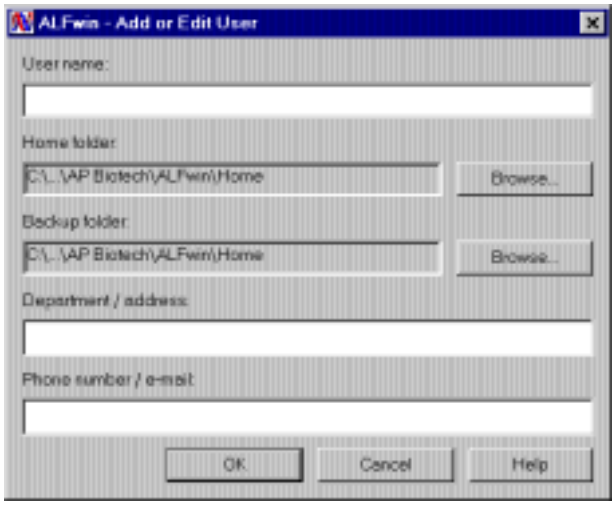

Figure 2-2. ALFwin - Add or Edit User dialogue.

2. Type your name in the **User name** field. You can use up to a maximum of 256 characters.

3. Your user and backup folders are created automatically when you enter a user name. Your home folder is automatically shown when you work with other ALFwin modules.

4. Click on **OK** to save the information and to return to the **ALFwin - Administrator** dialogue.

For further information on adding new users, see Section 3.2.

2.2.3 Linking ALFwin to the instrument

Define the connection of the instrument to ALFwin:

1. From the **ALFwin - Administrator** dialogue, highlight a **User** name and select an instrument in the **Available applications** field.

2. Click on **System Setup**. The **ALFwin - Edit System Settings** dialogue opens.

Figure 2-3. ALFwin - Edit System Settings dialogue.

The name of the instrument is shown in the top field of this dialogue. You can name your instrument here; the name is shown in the ALFwin Control Title bar during the run.

3. Type any comments in the **Comment** field.

4. Type the number of the COM port to which your instrument is connected.

5. Check that **Demo instrument** is disabled (unchecked).

6. Click on **OK**.

2.2.4 Starting ALFwin Control

Start ALFwin Control:

1. Select the instrument for your run in the **Available applications** field and click on **START**.

2. ALFwin Control starts.

3. A splash screen is shown for a few moments.

4. The information panel advises that initialization is taking place. If the instrument has been switched on since Control was started, the instrument program is loaded.

5. The information panel advises that the system is searching for the connection to the instrument.

6. The green and red indicator lamps on the ALF*express* II instrument remain illuminated and the three yellow indicator lamps flash in series.

7. When the connection has been established only the green indicator lamp remains illuminated on the instrument.

4. The **Tip of the Day** dialogue is superimposed on the ALFwin Control screen. This dialogue provides tips about using the program. Click on **Close** in this dialogue to proceed.

2.2.5 Mounting the gel cassette into the instrument

Leave the computer for a few moments and mount the prepared gel cassette into the instrument.

1. Lift the gel cassette and place its lower edge into the lower buffer reservoir.

2. Locate the two holes on the cassette on to the appropriately placed fixed screws protruding from the front of the instrument. The gel cassette should be pressed in against the two microswitches located on the front panel of the instrument. Check that the cassette is operating both of these switches. This is most easily seen by checking the indicator lamps on the front of the instrument since the yellow temperature lamp starts blinking when the microswitches are pressed in.

3. Check the water level on the right side of the instrument. The water level should be between the max and min marks of the water tube. If this is not the case, attach a small funnel to the water tube and add sufficient MilliQ water to bring the level up to the max mark.

4. Connect the two push-fit thermocirculator connectors of the instrument to the connectors on the gel cassette.

   The yellow temperature indicator lamp on the instrument flashes while pre-heat raises the temperature to the default 40°C. When the default temperature is reached, the yellow lamp illuminates continuously.

5. To switch on the laser and initiate the automatic laser alignment routine, close the lid of the instrument and open it again. While alignment proceeds, the yellow laser indicator lamp flashes. When alignment has been completed successfully, the laser indicator lamp illuminates continuously.

6. Check the laser beam for dust particles in the gel or on the glass in front of the detectors. If dust particles are in evidence, simultaneously adjust the vertical position of the gel cassette using the two adjustment wheels on the front panel of the instrument. Keep the gel level.

7. Fill the upper and lower buffer reservoirs with running buffer to the appropriate maximum level marks inside the reservoirs.

Figure 2-4. ALFwin control screen with Casebook dialogue open.

2.2.6 Entering information into a casebook

Casebooks are central to the operation of ALFwin Control as they contain the conditions for your run and also store details about the run and set up procedures for automatic post run actions. ALFwin is supplied with one casebook file called 'Sequence1st.cbk', which contains defined experimental conditions used making this first sequencing run. Casebooks are discussed in Chapter 4.

1. In ALFwin Control, select the command option **File:Open Casebook**. The **Open Casebook** dialogue is shown.

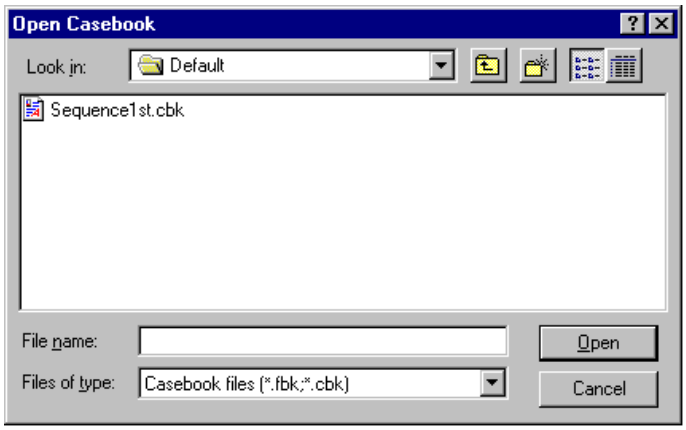

Figure 2-5. Open Casebook dialogue.

2. Browse to the **Default** folder and open it if the contents are not already displayed.

3. Select the file called 'Sequence1st.cbk', which contains the experimental conditions for this run.

4. Click on **Open**. The **File Information** tab of the **Casebook** dialogue is displayed.

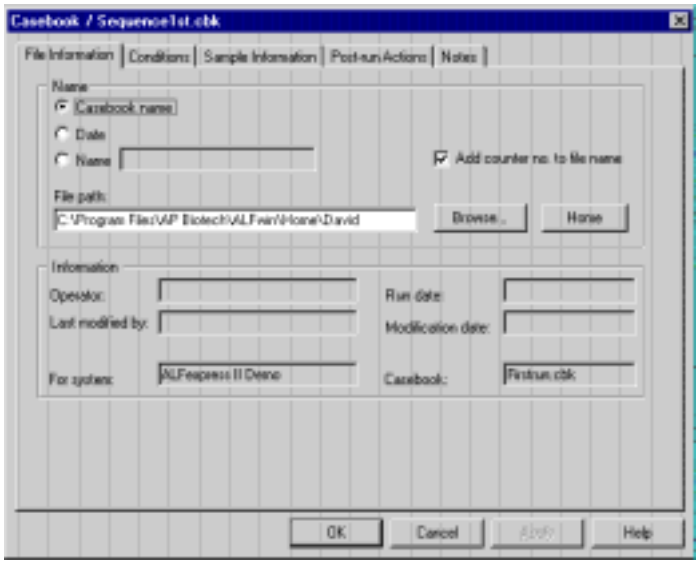

Figure 2-6. Casebook dialogue, File Information tab.

5. Enter a name for the result file by typing “Myresult” in to the **Name** field.

6. Check that the file path is correct. If necessary, click on the **Home** button to recall the location of your user folder.

7. Click on the **Conditions** tab. There you can see the experimental conditions that will be used for this run. No changes are necessary.

8. Click on the **Sample Information** tab and ensure that the **Clones** tab (in the lower left part of the dialogue) is selected.

   In the **Name** column, enter ‘M13’ for all ten clone positions. Add any comments in the **Comment** column.

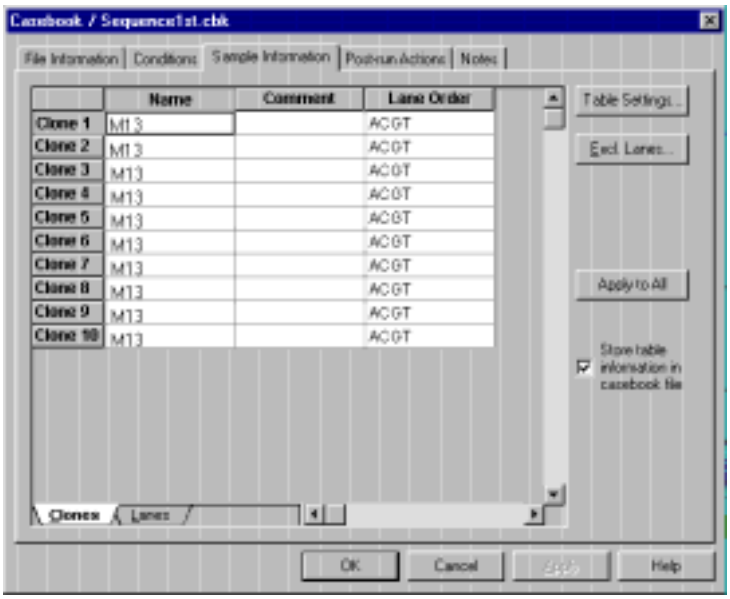

Figure 2-7. Casebook, Clones tab in Sample Information.

9. Click on the **Notes** tab

   Enter relevant notes in the **Pre-run notes** field.

.

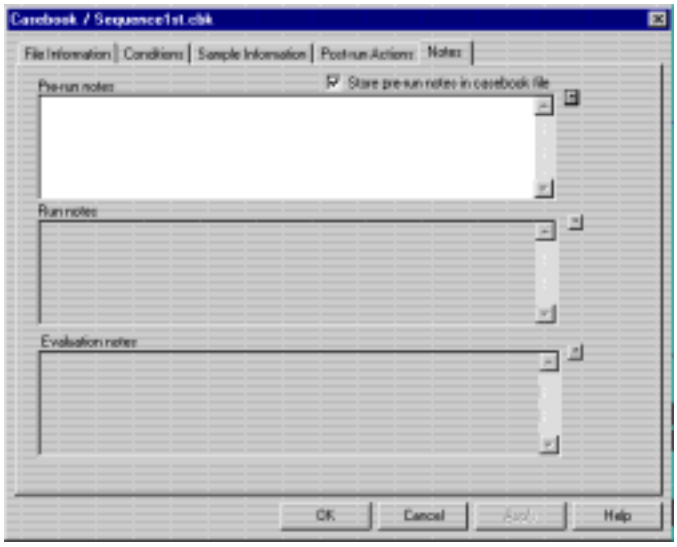

Figure 2-8. Casebook dialogue, Notes tab.

10. Click on **OK** in the **Casebook** dialogue. The dialogue closes and the **Save Casebook** dialogue is displayed.

    Click on **Save As** and the **Save Casebook As** dialogue is opened. Give a new file name to the casebook file (so that your colleagues can use the Sequence1st.cbk file), for example, 'Run1.cbk'. Click on **Save**.

2.2.7 Presetting the instrument

Before loading the samples and starting a run, the instrument temperature must be preset to the value defined in the casebook.

1. From the ALFwin Control toolbar on the screen, click on the **Preset** button. The temperature will now increase in the instrument to the value defined in the casebook.

2. Monitor the increase in **Temperature** in the Manual window of ALFwin control. The green value is the current temperature within the instrument. The black value is the one defined in the casebook.

3. Check that the yellow temperature indicator lamp on the instrument flashes while the temperature increases to the set value. When the temperature is reached, the yellow indicator lamp stays lit.

2.2.8 Denaturing the samples

We recommend that you use the Ready-To-Go® ALFexpress M-13 Ladder (order no. 27-4560-80), which you can obtain from your local Amersham Biosciences representative. There are four colour coded and labelled tubes, one for each of the four bases A,C,G,T. The samples should be stored at -20°C until use.

1. Denature the DNA samples at 95°C for 2-3 minutes; a thermocycler is recommended for this purpose.

2. Snap-cool the samples on ice for 2 minutes.

3. Pulse spin the samples in a microcentrifuge at 13000 rpm.

4. Return the samples to ice.

2.2.9 Loading the samples

You will now load the samples and make the final preparations of the instrument before starting the run.

1. Once the set temperature has been reached, indicated by the steady illumination of the yellow temperature indicator lamp.

2. Carefully remove the comb from the gel. Lift the comb straight out, avoiding bending in any direction.

Attention:
Use gloves to avoid skin contact with the gel.

3. Rinse out the wells very thoroughly with running buffer to remove any smear of non polymerised gel. Use a 50 ml syringe fitted with a 0.6 or 0.8 mm needle.

4. Prepare the Eppendorf Gelloader tips by manually shortening them by about 1.5 cm. Fit a tip to a micropipette (0-20 µl) and load 5 µl of the 'A' base sample to the first lane for each clone, i.e. lanes 1, 5, 9, etc.

5. Wash the tip in the upper buffer reservoir before taking each sample. Load the samples accordingly for each clone in the order of bases A, C, G, T.

6. Load gel loading buffer or "dummy" samples in the two outer lanes 0 and 41.

7. Fit the electrodes to each of the buffer reservoirs. The upper electrode has a black male lead that is plugged into the black female socket to the right of the mounted cassette, and the lower electrode has a red male lead that is plugged into the red female socket to the right of the mounted cassette.

8. Realign the laser by clicking on the **Realign Laser** button in the **Manual** window of ALFwin Control

9. Close the lid of the instrument.

2.2.10 Starting the run

You will now start the run.

1. Check that the laser and temperature indicator lamps on the instrument are continuously illuminated.

2. On the ALFwin Control screen, check the laser value and the temperature in the **Manual** window. The laser **Transmittance** value should have a reading over 70%.

3. Click on the **Start** button in the toolbar. The **Start Run** dialogue is shown. Check that the filename and path are correct and press on **OK**. A message is displayed on the screen:

   "The sampling will start after 16 seconds,
   please wait..."

4. A green lamp in the title bar and in the Windows 95 Taskbar confirms the run has started.

   The electrophoresis lamp on the instrument will be illuminated.

5. Wait until you can see curves in the **Curve** window to confirm the electrophoresis is running. The electrophoresis lamp should be continuously illuminated.

6. Follow the run in real-time in ALFwin Control.

   The result file is automatically generated at the end of the run and can be opened in ALFwin Sequence Analyser.

2.3 Analysing the results of your first run

At the end of the sequencing run, a result file was automatically created with the file name 'Myresult.alx'. This contains the raw data from the run in addition to a copy of the casebook which now includes the logbook and an Analysis log. ALFwin Sequence Analyser gives you a wide variety of useful analysis and presentation tools for improving data processing or the presentation of your results.

You will now perform an analysis of the run results in the ALFwin Sequence Analyser module. This involves a basic introduction to some of the major functions needed to analyse your first run to confirm that it was successful. Full description of the features mentioned, and many others besides, are detailed in Chapters 5 and 6 of this User Manual.

2.3.1 Starting ALFwin Sequence Analyser

To start ALFwin Sequence Analyser:

1. Restart ALFwin Administrator by locating and selecting **ALFwin Software** from the Windows 95 Start button menu options. The **ALFwin - Administrator** dialogue is displayed.

2. In the **ALFwin - Administrator** dialogue, select your user name or the name Default and then double click on **Sequence Analyser 2.00** under **Available applications**. The Sequence Analyser module is started as indicated by the splash screen.

3. The **Tip of the Day** dialogue is superimposed on the ALFwin Sequence Analyser screen. **Tip of the Day** provides tips about using the program. Click on **Close** in this dialogue to proceed further.

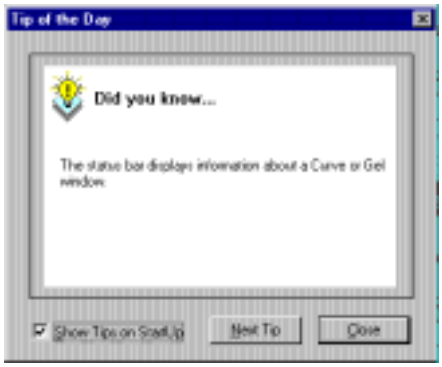
Tip of the Day
Did you know...
The status bar displays information about a Curve or Gel window.
Show Tips on Startup
Next Tip
Close

Figure 2-9. Tip of the Day dialogue.

2.3.2 Opening the result file

Open your result file:

1. Select **File:Open** or click on the **Open** button. The **Open** dialogue is shown.

2. In the **Open** dialogue, locate the 'Myresult.alx' result file. Select the file and click on **Open**.

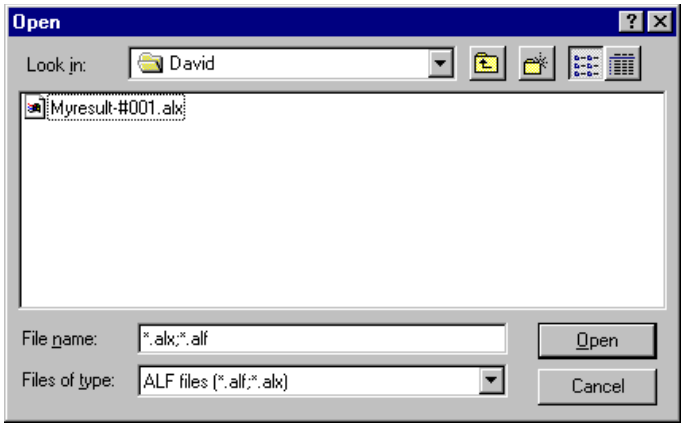

Figure 2-10. Open dialogue.

The **Result** window is displayed in the Sequence Analyser workspace.

The **Result** window contains a tree structure of all the clones contained in the opened result file. All clones in this result file have the raw data icon.

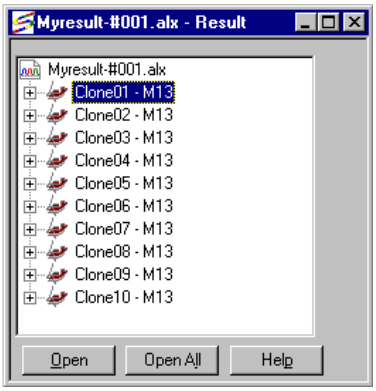

Figure 2-11. Result window.

3. To view the lane information for a clone, click on the **+** symbol beside the clone. Conversely, to hide lane information from view click on the corresponding **-** symbol.

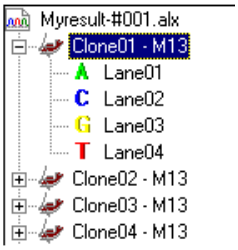

Figure 2-12. Result window, lane information.

2.3.3 Processing the data

1. Select **Analyse:Process**. The **Process Status** dialogue opens.

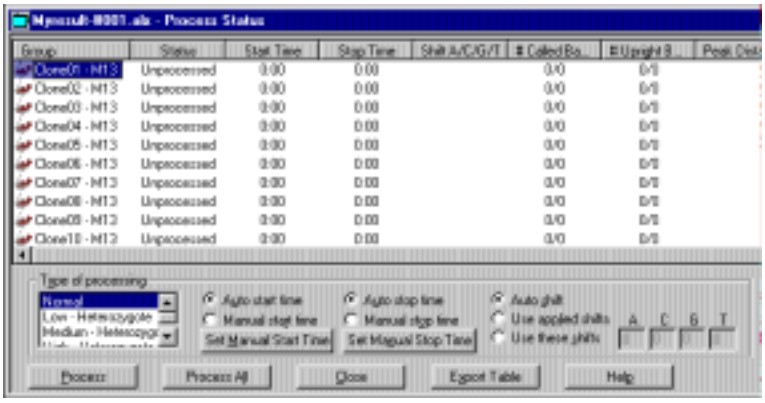

Figure 2-13. Process Status dialogue.

2. Click on **Process All.**

3. When processing is complete, observe the following:

- The icon to the left of the clone name changes to the processed data icon if the processing is successful.
- The **Status** column shows **Normal** type of processing (no heterozygocity expected).
- The **Start Time** and **Stop Time** columns show the times where the processing of data starts and stops.

- The **Shift A/C/G/T** column shows **Auto**, indicating that automatic correction for smiling[1] has been applied.
- The **Upright bases/Ambiguities** column contains two figures separated by an oblique stroke. The first figure shows the reading length of the sequence for each clone.
- The **Called bases/Ambiguities** column contains two figures separated by an oblique stroke. The first figure is the reading length and this number includes a further 300 bases beyond the point where the certainty of the result fell below 99%. The second figure, to the right of the oblique stroke, is the number of ambiguities. These are the positions where the base calling algorithm had two or more alternative interpretations.

2.3.4 Analysing the processed data

There are several analysis options that are open to you to confirm the quality of the results. Just some of these options are described here, although as you become experienced with using ALFwin Sequence Analyser, you will discover a wide range of analysis and presentation possibilities to get the most out of your data (see Chapters 5). Examples of analysis options include:

- checking the upright bases figure. The same M13 sample was loaded across the gel and there should be no great disparity between adjacent clones.
- checking for excessive shifts
- checking the signal levels of the raw data

You can also:

- confirm the stability of the instrument readings (see below)
- check the sequence alignment (see below)

Confirming stable instrument readings

The instrumental parameters are logged during a run and can be displayed as curves.

1. Click on the **Curve** window to make sure it is activated or open the curves for the appropriate clone.

1. "Smiling" is the phenomenon produced when the samples in the first and last lanes move more slowly than those towards the centre of the gel.

2. Select **View:Properties** and click on the **View** tab in the **Curve Properties-Layout** dialogue.

3. Select the required **Instrument data**. You can select an individual item, specific items if you hold down the <Ctrl> key while you select them, or a range of items if you press the <Shift> key while selecting the last item.

4. To effect the changes without closing the dialogue, click on **Apply**. To close the dialogue and effect the changes, click on **OK**.

5. Place the mouse pointer on the **Instrument data** curve of interest. The value at the pointer is shown in the Status Bar.

Checking the sequence alignment

It is possible to align the sequences within open runs. This presents several useful analysis possibilities and functions such as:

- comparing sequences from different files
- checking for base-specific differences between clones
- calculating the consensus sequence
- displaying amino acids.

This powerful feature is not described here, but instead in Chapter 6.

2.3.5 Other options

You can:

- edit the sequence data
- check the gel view
- search for specific sequences
- display complementary and reverse variants of your sequence
- print all of your data
- export the sequence for further analysis in other application software

2.3.6 Viewing casebook information and adding analysis notes

The casebook information is included in the result file saved at the end of a run. In the casebook, you can:

- read the result **File Information**
- read the **Run conditions**
- read, add or change **Sample Information**
- read the Post Run Action settings
- read the **Pre-run** and **Run notes**
- write **Analysis notes**
- read the **Logbook** and **Analysis Log.**

To view or edit the Casebook:

1. Select **Edit:Casebook** or click the **Edit Casebook** button. The **Casebook** dialogue is shown.

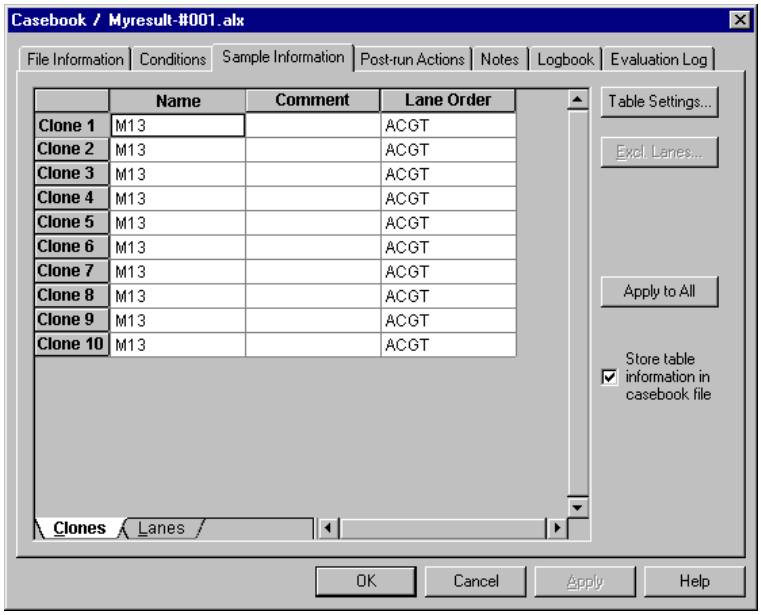

Figure 2-14. Casebook dialogue, Sample Information tab.

2. Select the **Sample Information** or **Notes** tabs to change or add information in the Casebook.

3. Use the remaining tabs to access read-only information in the Casebook.

4. Click on **OK** and the changes and additions will be saved when you save the result file.

2.3.7 Printing a report

A report is a print out which can include items from the casebook, curves, sequences and headers.You select the items you want to include by means of the **Report Settings** dialogue. The settings can be saved as a layout file which allows you to recall a report configuration. Specific report options and layouts are described in Chapter 5.

1. Select **File:Report**. The **Report Settings** dialogue is shown.

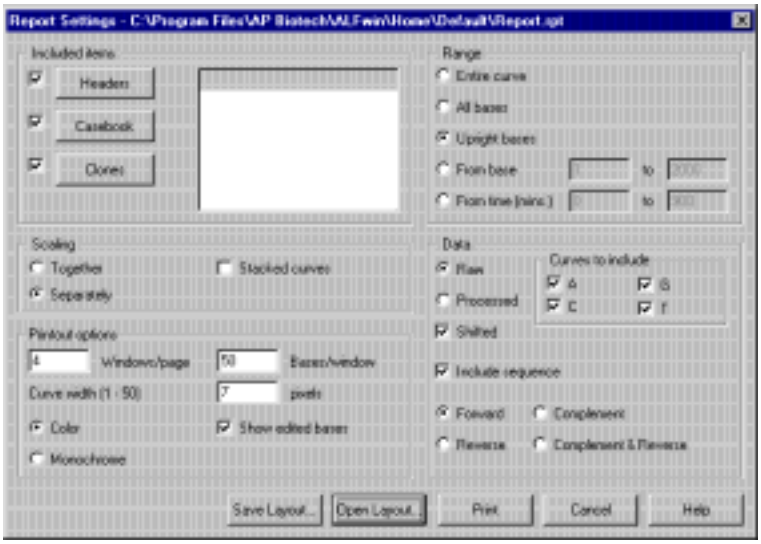

Figure 2-15. Report Settings dialogue.

2. Click on **Print** to send the report with default settings to the printer.

2.3.8 Saving the whole result file

To save the result file in the same location and using the existing file name, select **File:Save**.

To save using a different name or location:

1. Select **File:Save As**. The **Save As** dialogue opens.

2. Use the dialogue to select the folder where the file is to be saved and enter a name for the file.

3. Click on **Save**.

Congratulations!

You have now completed your first run.

3 ALFwin Administrator

ALFwin Administrator is used to create your user name, which can then be used to open the ALFwin Control or Sequence Analysis modules. When you create your user name, additional information is given such as the storage and backup storage folders for result files. ALFwin Administrator is used to connect one or two ALF family instruments or demonstration (virtual) instrument(s) to the computer for simultaneous control capability. This chapter is divided into the following sections:

3.1 Starting ALFwin Administrator

3.2 Creating a user name and folder

3.3 Connecting an instrument to ALFwin Control

3.4 Launching the ALFwin applications

3.1 Starting ALFwin Sequence Analyser

To start ALFwin Administrator, locate and select **ALFwin software** from the Windows 95 Start button menu options. The **ALFwin - Administrator** dialogue is shown.

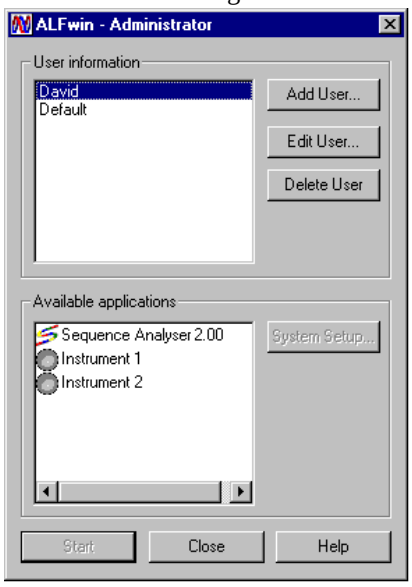

Figure 3-1. ALFwin Administrator dialogue.

Note: If you have also obtained the DNA fragment separation software from Amersham Biosciences, you will also see an option called **Fragment Analysis 1.00** in the **Available applications** field. For further information on DNA fragment separation, please refer to the ALFwin Fragment Analyser 1.00 User Manual or contact your local Amersham Biosciences representative.

3.2 Adding a user

To make it easier to administrate documents and files, you should create your own user name, user folder and backup folder. The folders are used to store the casebook and run files, including layout files, report files and alignment item files that are created during use of the ALFwin Software.

When you install the software into your computer, a Default user and Default folder are created. The Default folder contains some useful standard routines which you can copy into your own folder using Windows Explorer. If you use the normal installation routine, the Default folder can be found in c:\Program Files\AP Biotech\ ALFwin\Home. Normally you use this same location for your own user files.

The facility for creating a backup file allows you to save a copy of the result file that is generated at the end of a run in alternative drive location, usually on a network server.

3.2.1 Adding a new user

Adding a new user allows you to specify your user name and thus a location for your user and backup files.

1. From the **ALFwin - Administrator** dialogue, click on **Add User**. The **Add or Edit User** dialogue is shown.

2. Type your name in the **User name** field. You can use up to a maximum of 256 characters.

3. Your default **Home Folder** is created automatically when you enter a user name. It has the same name as the user and is located in the Program Files\AP Biotech\ALFwin\Home\'user name' folder.

4. Your **Backup Folder** is also created automatically when you enter a user name.

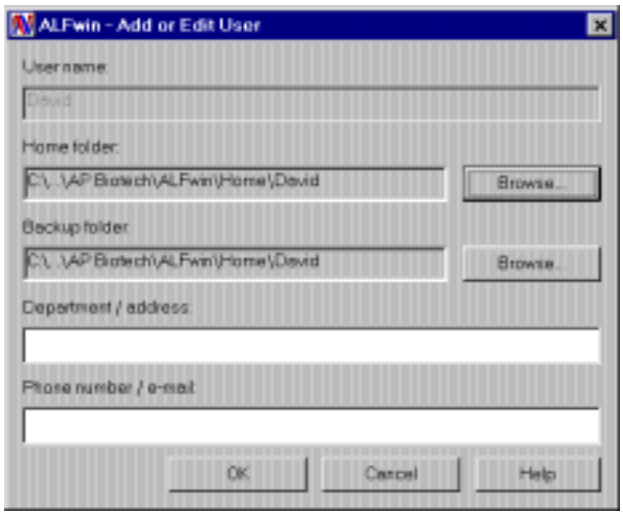

Figure 3-2. Add or Edit User dialogue.

5. If you want to change the location of your home folder, click on the **Browse** button adjacent to the home folder field. The **Browse for folder** dialogue opens.

6. Select the path to an existing folder or use the **Create New Folder** button to generate any new folders you require. To open the new folder, double click the folder name or select the name then click on **Open**.

7. Click on **Save** to create your home folder. The **Add or Edit User** dialogue returns.

8. If you wish to change the name or location of your **Backup Folder**, click on the **Browse** button adjacent to the backup folder field and use the **Browse for folder** dialogue to select a path and name for your backup folder. Use the **Create New Folder** button to generate any new folders you require. Choose, if possible, an alternative drive for this backup folder but be aware that a diskette may not have sufficient capacity for the files created by a run.

9. If you require, enter information in the **Department/Address** and the **Phone Number/E-mail** fields.

10. Click on **OK** to save the information and to return to the **ALFwin - Administrator** dialogue.

If you have completed the use of the **ALFwin - Administrator** dialogue to add or edit user information, you can proceed directly into ALFwin Sequence Analysis by selecting it in the **Available Applications** field. To move into ALFwin Control, you must first specify the connected instruments as described in Section 3.3.

3.2.2 Editing a user

You can edit:

- the location of the home and backup folders
- information in the **Department/Address** and **Phone Number/E-mail** fields.

To edit a user:

1. From the **ALFwin - Administrator** dialogue, select the user name and click on the **Edit User** button. The **Add or Edit User** dialogue is shown.

2. Edit the **Department/address** and **Phone number/e-mail** fields.

3. The **Home folder** and **Backup folder** name fields have a dark background to indicate that they can not be edited by typing. Instead, click on the adjacent **Browse** button. to open the **Browse for folder** dialogue.

4. Use the dialogue to select the path for your folder. Use the **Create New Folder** button to generate any new folders you require.

5. Click on **OK** to complete the edit and to return to the **ALFwin - Administrator** dialogue.

3.2.3 Deleting a user

1. From the **ALFwin - Administrator** dialogue, select the user name you want to delete.

2. Click on the **Delete User** button. A dialogue asks you to confirm the deletion.

3. Click on **Yes** to confirm the deletion.

Folders and files are not deleted by this command.

3.3 Connecting an instrument to ALFwin Control

To make a sequencing run it is necessary that the software is connected to at least one ALF family instrument. After the connection is made, an identification can be added for the serial (COM) port on the back of the computer to which an instrument is connected. Two instruments can be connected and used simultaneously.

Trace functions can be initiated by ALFwin Administrator which allow service technicians to examine the operation of the instrument control.

3.3.1 Connecting an ALF family instrument

To define the connection of an instrument:

1. From the **ALFwin - Administrator** dialogue, highlight a **User** name and select an instrument in the **Available applications** field.

2. Click on **System Setup**. The **ALFwin - Edit System Settings** dialogue opens.

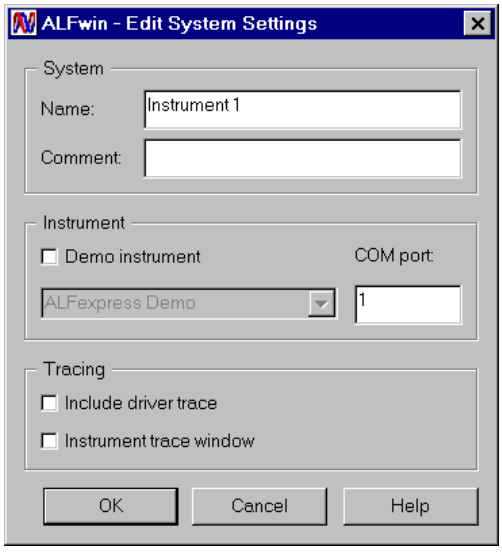

Figure 3-3. Edit System Settings dialogue.

3. The name of the instrument is shown in the top field of this dialogue. You can name your instrument here; the name is shown in the Title bar of ALFwin Control during a run. If you wish to change the name, select it and type in the name you require. If you include a name or ID for the connected instrument, it will help you to remember which instrument is connected to each COM port.

4. Type any comments in the **Comment** field.

5. Type the number of the COM port to which your instrument is connected. The default at installation is COM port 1 to instrument 1 and COM port 2 to instrument 2.

6. Click on **OK**. An instrument is connected to the software.

7. Select the second instrument, if required, and proceed as above to connect it to the second port on the computer.

Note: If required, a service engineer can select the **Instrument Trace Window** option to view information about communication between the instrument and the program. The trace window resides on the Windows 95 taskbar. If the instrument trace information is to include information from the instrument driver the **Include Driver Trace** option should be selected.

3.3.2 Connecting to the demonstration instrument

A demonstration run can be performed by connecting a demonstration instrument which operates as a virtual instrument. The benefit of being able to connect a demonstration instrument is the ability to run the software for learning or demonstration purposes without the need for a real instrument to be connected to the computer.

To install the demonstration instrument:

1. From the **ALFwin - Administrator** dialogue, select an instrument and click on **System Setup** to open the **ALFwin - Edit System Settings** dialogue.

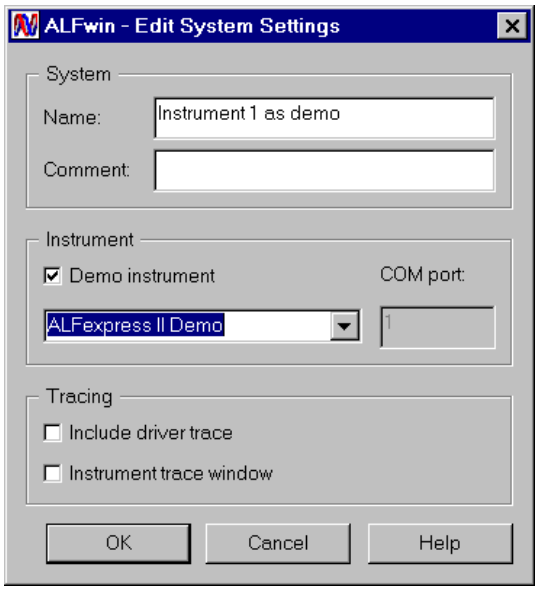

Figure 3-4. Edit System Settings dialogue.

2. Check the **Demo instrument** option box.

3. Select **ALFexpress Demo** or **ALFexpress II Demo**.

4. Click on **OK** to return to the **ALFwin - Administrator** dialogue.

5. Click on **OK** to start ALFwin Control with the demo instrument connected.

3.4 Launching the ALFwin applications

ALFwin Control and Sequence Analyser are launched directly from ALFwin Administrator.

3.4.1 Launching ALFwin Control

1. To open ALFwin Control, select the instruments in the **Available applications** field and click on **Start**.

2. Control is launched and a splash screen is shown for a few seconds.

3. An information panel advises that initialisation is taking place. If the instrument has been switched on since ALFwin Control was started, the instrument program is loaded.

4. An information panel advises that the system is searching for the connection to the instrument.

5. ALFwin Control is launched.

Note: You can also launch ALFwin Control, from ALFwin Sequence Analyser (see Chapter 5).

3.4.2 Launching ALFwin Sequence Analysis

1. From the **Available applications** field of the **ALFwin - Administrator** dialogue, select **Sequence Analysis 2.00**.

2. Click on **Start** to launch the application.

4 ALFwin Control

ALFwin Control is used to control sequencing runs using one or two ALF family instruments. While a run is being performed, the data is monitored in real-time allowing you to confirm that the run is proceeding normally.

All important information needed to perform a run in ALFwin Control is specified within the casebook. A casebook specifies the experimental run conditions that are used to control the instrument. You can make changes to the run conditions before or during a run. Any changes you make are automatically recorded in the logbook. The casebook stores important information about your samples and allows you to define the storage folder for the generated result files. At the end of a run, defined procedures called post-run actions are automatically executed.

A typical run consists of the following steps:

Start ALFwin Control	You start ALFwin Control from ALFwin Adminstrator or Sequence Analyser.
Use an existing casebook file or create a new casebook. You can create your new casebook from an existing casebook file.	You name your result file, specify the run conditions, add sample information, specify data storage folders, add pre-run notes, and specify any automated post-run actions.
Preset the instrument and start the run	You can preset the temperature of the instrument prior to starting the run.

Perform any run activities	You can monitor the run on the screen as it proceeds and check the amplitude of the curves. You can perform other activities such as: • enter sample information, standard settings and other analysis settings in the casebook • include run notes in the casebook • manipulate the screen layout for presentation purposes • pause the run • start another run on a second instrument. • analyse pre-result data
End the run	The run ends automatically or you can end the run manually.

This chapter is divided into the following sections:

4.1 Starting ALFwin Control

4.2 Introducing the ALFwin Control interface

4.3 Working with casebooks

4.4 Modifying casebook parameters

4.5 Starting a run

4.6 Activities that can be performed during a run

4.7 After the run is finished

4.8 Changing the screen layout

4.9 Exiting ALFwin Control

4.1 Starting ALFwin Control

ALFwin Control is normally started from ALFwin Administrator. You can also move into Control from ALFwin Sequence Analyser.

4.1.1 Starting ALFwin Control from ALFwin Administrator

1. From the Windows 95 Start button menu options, select **Programs:ALFwin software**.

2. Use the **ALFwin Administrator** dialogue to select your user name from the list provided. Please refer to Section 3.2 if you need to add or edit a user.

3. Select an instrument in the **Available applications** field. The colour of the icon in front of the instrument name indicates the current status:

Grey	Unused system/instrument
Yellow	Instrument connected to Control but not running
Green	Instrument connected to Control and running

Figure 4-1. ALFwin Administrator dialogue.

If you need to set up the link between the computer and the instrument, please refer to Section 3.3

4. Click on **Start**. The ALFwin Control splash screen is shown for a few moments. You can close this splash screen more quickly by clicking on it or by pressing any key.

5. If you selected the demonstration instrument from ALFwin Administrator, a warning is shown advising how you must proceed to reconnect a real instrument. To continue with the demonstration instrument, click on **OK**.

6. If a real instrument is connected and switched on, the following messages are displayed:

"Initializing"	program looks for the COM port,
"Looking for instrument"	searches for the instrument,
"Loading instrument program"	loads the instrument program to the instrument,
"Connecting to the instrument"	connects to the instrument.

7. The main **ALFwin Control** screen opens with a superimposed **Tip of the Day** dialogue that provides tips about using the program. To view more tips, click on the **Next Tip** button. If you do not want to see the dialogue at each startup, uncheck the **Show Tips on StartUp** box. To view the **Tip of the day** dialogue again, select **Help:Tip of the Day**.

Note: This dialogue must be closed before you proceed further with Control. Click on **Close**.

4.1.2 Starting ALFwin Control from Sequence Analyser

1. Select **File:Start Instrument**

2. Use the **ALFwin - Select System** dialogue to select the instrument

3. Click on **Start** to enter ALFwin Control.

4.1.3 Using two instruments

ALFwin Control can simultaneously run two instruments from one computer. The instruments are distinguished by the computer's COM port to which they are connected. Connection with the instruments is

established automatically when you start ALFwin Control and the software identifies the type of instrument connected so that screen contents and control procedures can be adjusted accordingly.

If a computer is being used to control two instruments, ALFwin Control must be started twice. Both instances reside on the taskbar. One instance controls Instrument 1, the other Instrument 2.

To connect a second instrument, repeat the steps described in Section 4.1.3, selecting the second instrument at Step 3.

4.1.4 Automatic settings for ALFexpress family instruments

If you start ALFwin Control when using an ALF*express* II or ALF*express* analyser with a gel cassette in place, the following parameters are set automatically:

- the temperature of the gel is preset to 40 °C and the temperature begins to increase until this value is reached
- the light on the panel is switched on when the lid is open.

Moreover, depending on which of the two analysers you are performing a run, the following also apply:

ALF*express* II	ALF*express*
the laser is switched on and aligned when you press the **Realign Laser** button in the Manual window of ALFwin Control (see Section 4.5.1)	the laser is switched on and adjusted after you have closed and opened the lid on the instrument
the run conditions can be stepped or ramped to different values during the course of the run (See Section 4.4.2)	the run conditions can be stepped to different values during the course of the run

4.2 Introducing the ALFwin Control interface

The ALFwin Control interface contains the **Curve View**, **Manual** and **Logbook** windows together with the **Title**, **Menu**, **Tool** and **Status** bars.

There is also a run light indicator in the Windows 95 taskbar.

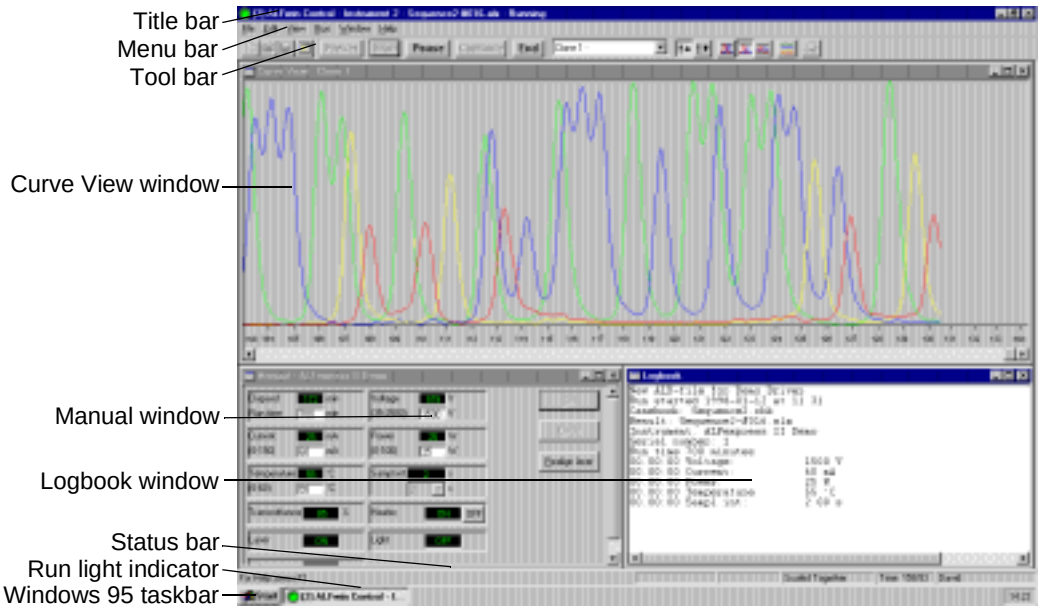

Figure 4-2. ALFwin Control screen controlling a run on an ALF*express* II instrument. Note that you can maximize the display of any window or change the size of the windows by dragging the window borders. The fonts size of the displayed texts may also be reduced to display more information.

4.2.1 Windows

The following windows are visible:

The **Curve View** window	This provides a real-time graphical view of the lanes from the run.
The **Manual** window	This shows the experimental setup conditions in black and the present measured values in green.
The **Logbook** window	This logs all information pertaining to the current run.

4.2.2 Bars

The following bars are available:

Title bar	This shows information about the instrument to which ALFwin control is

connected, the name of the open casebook or result file and the status of the run.

Menu bar	Click on any menu option to view and select the specific commands.
Toolbar	The toolbar buttons allow you to quickly implement the most commonly used commands. The range of active buttons is dependent upon the currently active window in the Control workspace. Unavailable buttons are greyed out. Use the mouse pointer to get a description of each button. You can hide the toolbar by deselecting (unchecking) the active **View:Toolbar** command.

Status bar	This gives you detailed information about, for example, the position of the mouse pointer and current mode selected on the screen. You can hide the status bar by deselecting (unchecking) the active **View:Status Bar** command.

4.2.3 The light indicator

The light indicator provides a quick visual check of the operation of the instrument and is visible in the Windows 95 taskbar and in the ALFwin Control title bar.

Grey light	The instrument is not running but is connected.
Green light	The instrument is running.
Red light	The run is paused or performing post-run actions.
Grey light with red cross	ALFwin Control is not in contact with the instrument.

4.3 Working with casebooks

4.3.1 Introducing casebooks

A casebook is needed to perform a run. A casebook is a file that contains the parameters for the experimental conditions used by ALFwin Control to control the run. The casebook also defines the storage locations of results and allows various data to be stored such as sample information and experimental notes. Moreover, various post-run actions can be selected, which are procedures that are automatically executed at the end of the run.

Casebook files (.cbk)

A new casebook file can be created within ALFwin Control for each run that you want to perform. However, you may find it more useful to open an existing casebook file and use it directly or make some appropriate modifications according to the current experiment. Casebook files must be saved before starting a run. By saving modified casebooks under new file names, you are able to build up your own library of casebooks tailored to your own application requirements. ALFwin Sequence Analyser 2.00 is supplied with a default casebook, 'Sequence1st.cbk', located in the Default folder. Run conditions (voltage, current, power, sample interval, temperature and time) for different experimental applications can be obtained from the Recommended Operating Procedures located on the internet page, *www.amershambiosciences.com*.

Casebook contents

The contents of the casebook file are separated into several tabs:

File Information — After a run, the data, logbook and a copy of the casebook are saved as a result file. This tab allows you to specify the name and location of the result file so that saving can proceed automatically.

Conditions — These are the run settings for the ALF family instrument controlled by ALFwin Control. These settings specify the run time, gel temperature, the voltage, current and power limits and the sampling interval. These settings can be automatically stepped or ramped to different values during the course of a run.

Sample Information	This tab allows you to store information about the clones and the lanes in the gel.
Post-run Actions	This tab allows you to specify activities that will proceed automatically when the run is completed. These include data processing, export, printing a report, launching tools and file backup.
Notes	You use the casebook to make your pre-run, run and analysis (post-run) notes. The casebook manages note taking, allowing access only to the appropriate type of note according to the stage reached in the experimental procedure.

Casebook files during a run and result files after a run

The casebook can be fully modified *before* the run is started and the modifications are saved in the casebook file. You can continue to edit some of the information in the casebook *after* the run has been started although these are *not* saved in the casebook file. Changes made after the run has been started are saved in the result file (.alx) that is generated at the end of the run. A result file contains:

- a copy of the casebook information including any information that you edited during the run
- logbook information from the Logbook window
- the raw data obtained from the run
- an analysis log (empty until an analysis is performed)

This information can be accessed in ALFwin Sequence Analyser.

4.3.2 Opening an existing casebook

ALFwin is supplied with one standard casebook file appropriate for making your first sequencing run (see Chapter 2). You may run this directly or else modify it to suit your own run requirements. Remember to contact your Amersham Biosciences representative or visit the internet page, *www.amershambiosciences.com* to obtain run conditions for different experimental applications.

To open an existing casebook file:

1. From the **ALFwin Control** window, select **File:Open Casebook** or press the <Ctrl+O> keys or click on the **Open Casebook** button. The **Open Casebook** dialogue is shown.

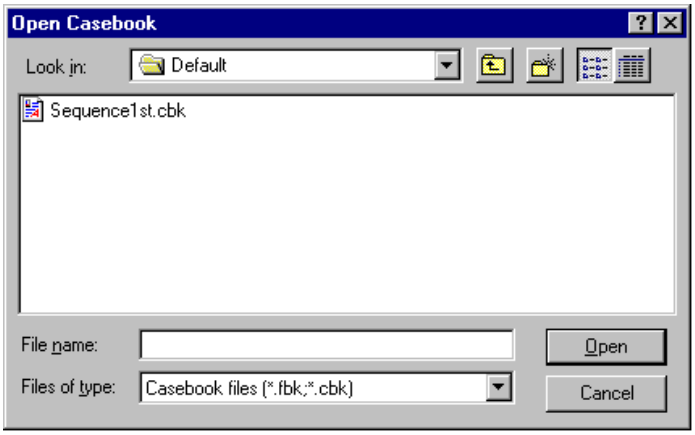

Figure 4-3. Open Casebook dialogue.

2. Use this dialogue to navigate the filing system and select a casebook file. Casebooks files for sequencing are always saved with the file extension, .cbk.

Note: If you also have ALFwin Fragment Analyser 1.00 installed on your computer, you will see that it is possible to select casebook files for the control of sequencing runs, as denoted by the .fbk file extension; *do not* select these casebook files for sequencing runs.

3. Double-click the selected .cbk file or select it and click on **Open**. The **Casebook** dialogue is shown with the name of the chosen casebook at the top.

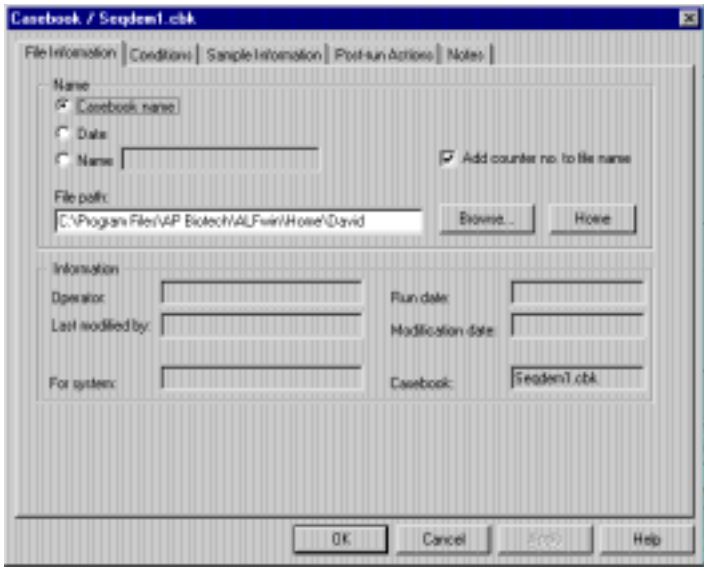

Figure 4-4. Casebook dialogue, File Information tab.

Note: You can open a casebook that you have used recently by selecting it from the list at the bottom of the **File** menu. The four latest saved files are shown.

4. If you want to use the casebook without modification, click on **OK**. This will close the **Casebook** dialogue and load it into ALFwin Control.

   If you want to modify the casebook, refer to Sections 4.4. When you have performed all of the modifications of the casebook click on **OK**. This will close the **Casebook** dialogue.

5. The **Save Casebook** dialogue opens (see Section 4.3.4 for details).

4.3.3 Creating a new casebook

ALFwin Control allows you to create a new casebook by supplying an unnamed casebook file with default parameters set for the instrument. To create a new casebook file:

1. Select **File:New Casebook** (<Ctrl+N>) or click on the **New Casebook** button. The **Unnamed Casebook** dialogue with default values for the appropriate instrument is shown.

Note: If you have *both* ALFwin Sequence Analyser 2.00 and ALFwin Fragment Analyser 1.00 software applications installed on your computer, the **Select Application** dialogue will be displayed. Click on **Sequence Analyser** and click on **OK**.

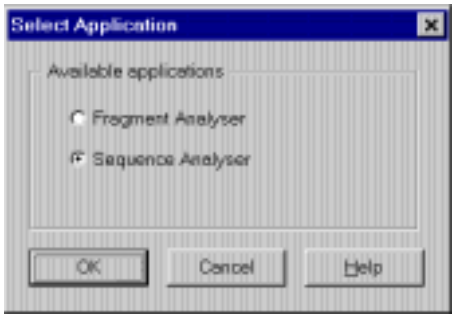

Figure 4-5. Select Application dialogue.

2. Edit the new casebook according to the procedures described in Section 4.4.

4.3.4 Saving the casebook

You must close the **Casebook** dialogue before you start a run. If you have created a new casebook file or opened and modified an existing casebook file, it must be saved when closing the **Casebook** dialogue.

To close and save the **Casebook** dialogue:

1. Click on **OK** in the **Casebook** dialogue. The **Save Casebook** dialogue is shown.

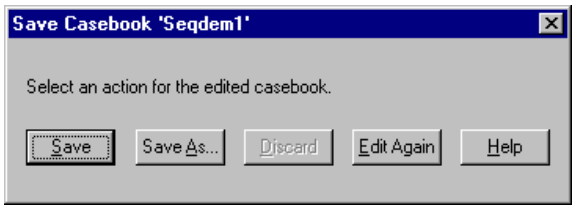

Figure 4-6. Save Casebook dialogue.

2. Select from the options available:

Save	To save the casebook under the existing name and folder.

Save As	To save the casebook with a new name and/or folder. Selecting this option opens the **Save Casebook As** dialogue. Use this dialogue to specify a name and location for the casebook or to rename an existing casebook that has been edited. This is recommended when modifications have been made to an opened casebook.
Discard	Available only after creating a new casebook. This option removes the new casebook without saving or loading it.
Edit Again	To leave the save procedure and return to the **Casebook** dialogue.

4.3.5 Editing a casebook before a run

As long as you have not started the run, you can make changes to the casebook. Changes made before the run is started are saved in the casebook file (.cbk file). After the run has been started, you can still change some items such as:

- post-run action settings,
- contents of the sample information table,
- run notes.

Any changes that are made during or after the run are saved in the result file (.alx file).

To edit the casebook:

1. Select **Edit:Casebook** or click on the **Edit Casebook** button. The **Casebook** dialogue is shown.
2. Make the changes (see Section 4.4).
3. Click on **OK**. The **Save Casebook** dialogue is shown.
4. Click on **Save**.

Note: If you edit a casebook during the run, you are warned that the changes are only saved in the result file. You can switch this warning off by selecting **Don't show this warning in the future** and clicking on **OK**.

4.3.6 Printing a casebook

A casebook can be printed before starting a run:

1. Complete the editing of the casebook prior to starting a run. Select **File:Print Casebook** or press the <Ctrl+P> keys or click on the **Print Casebook** button. The **Print Casebook** dialogue is shown.

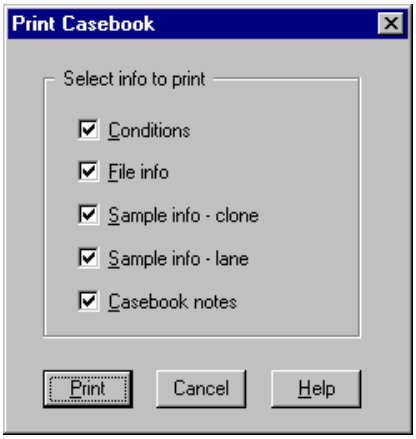

Figure 4-7. Print Casebook dialogue.

2. Select the parts of the casebook to be printed.

3. Click on **Print**. The **Print** dialogue is shown.

Note: You can also print out the casebook once the run has ended and the result file has been automatically saved by selecting **File:Report** in Sequence Analyser (see Section 5.12).

4.3.7 Print Setup

Printing is made according to the print setup parameters that you have defined for the current printer in Windows 95. You can alter the print setup by select Settings:Printers from the Windows 95 Start button. In the **Printers** dialogue, select the appropriate printer icon and select **File:Properties**. Make the appropriate selections.

Note: The same print setup is used to print the post-run report.

4.4 Modifying casebook parameters

The **Casebook** dialogue is divided into five tabs, each containing parameters that may be modified before a run and some modified during a run. Modifications that are made before a run is started are saved in the casebook file (see Sections 4.3.4 and 4.3.5) and changes made during a run are stored in the result file. The five tabs are:

- **File information** (see Section 4.4.1)
- **Conditions** (see Section 4.4.2)
- **Sample information** (see Section 4.4.3)
- **Post-run Actions** (see Section 4.4.4)
- **Notes** (see Section 4.4.5)

4.4.1 File Information tab

The information in this tab specifies the name and location of the result file (extension .alx) so that saving can proceed automatically during the run. This section also stores operator and date information. You have the option of changing the default result file name before the run is started.

A result file contains the following:

- raw data
- a copy of the casebook file used
- the logbook
- the analysis log (empty until an analysis is performed in Sequence Analyser)
- data from post-run processing

1. Select the **File Information** tab in the **Casebook** dialogue.

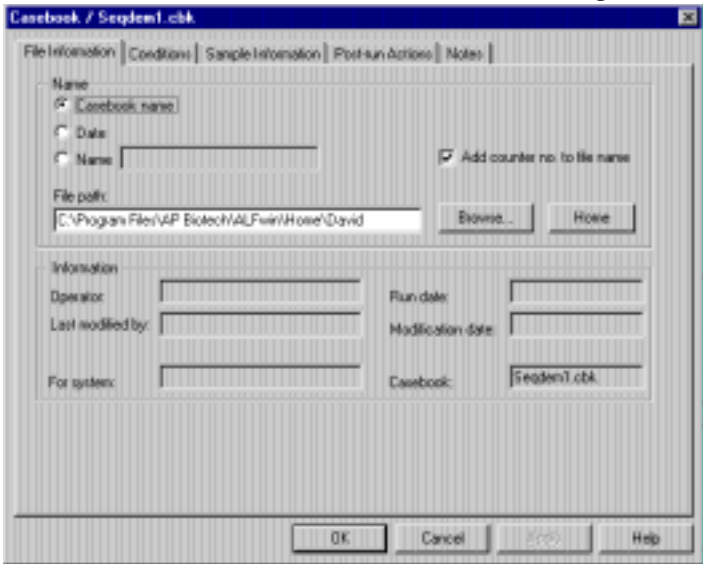

Figure 4-8. Casebook dialogue, File Information tab.

Use this dialogue to enter the name and location of the result file.

2. Select the **Name** of the result file. You can name the file according to:

Casebook name	This is the default setting and uses the casebook name.
Date	Use the current date.
Name	Use the name typed into the **Name** field.

3. By default, each file name includes the casebook name and a counter number. The counter number increases with each successive result file that you produce. This ensures that previously created files are not over-written. If the number passes 999, it will begin again at 1 and you may have to move, rename or delete files with low serial numbers. The counter is user specific and independent of what name you select on your result file. The counter will only increase when it is on. To disable this auto-numbering function, deselect the **Add counter no. to filename** button. ALFwin will warn you if you then select a filename that will overwrite an existing file.

4. Edit the **File path** field if you want to change the folder where the result file is stored. If you want to be in your home folder, click on **Home** and the path to your home folder will be automatically given. Alternatively, use the **Browse** button to open the **Select Directory** dialogue and use it to locate the destination folder for the result file. Remember that you can create a new folder in this dialogue using the standard Windows 95 functions. Click on **Open** to complete and close this dialogue.

4.4.2 Conditions tab

The information in this tab specifies the run settings for the ALF family instrument controlled by the computer when the run starts, and the settings able to be set are dependent on the instrument connected. These settings specify the run time, temperature, the voltage, current and power limits and the sampling interval. These settings can be programmed to change during the course of a run. You must complete any editing of run conditions in the casebook before you start a run.

Editing run conditions

1. From the **Casebook** dialogue click on the **Conditions** tab.

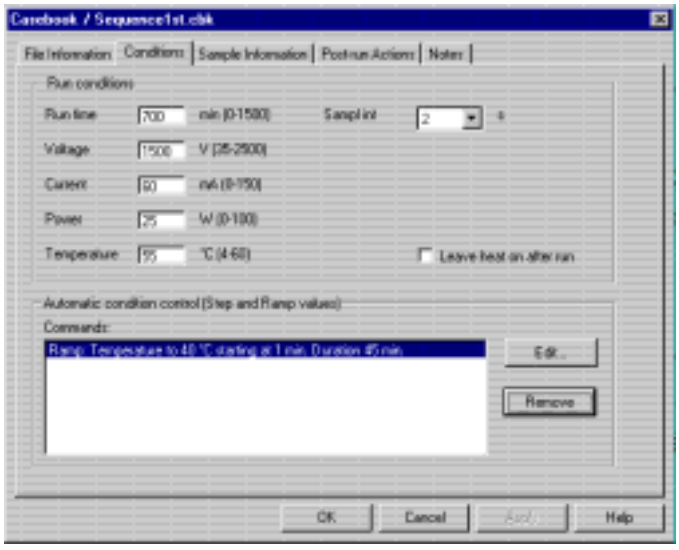

Figure 4-9. Casebook dialogue, Conditions tab.

2. Edit the run conditions as required. You can use the <Tab> key to move between fields.

Note: The **Voltage**, **Current** and **Power** settings specify the *maximum* values that can not be exceeded during the run. It is usual to select a limiting value for one of the three settings, for example **Power**, and to specify high non-limiting values for the other two settings.

Voltage	The maximum electrophoresis voltage.
Current	The maximum current in milliAmperes.
Power	The maximum power in Watts.
Run time	The time of the electrophoresis run in minutes.
Temperature	The thermostatically controlled water temperature in degrees centigrade.
Sample int	The interval between data samples in seconds.
Leave heat on after run	Select this option when you plan to start a new run soon after the current run.

Activating stepped or ramped pre-set changes for ALF*express* II

If you are making a run using ALF*express* II you use the **Automatic condition control** to set a stepped change to the set conditions during the course of the run or to introduce a ramped change in conditions. A ramped change differs from a stepped change in that the change is made gradually over a specified period of time. You can preset a change in the:

- electrophoresis voltage
- current
- power
- temperature

To set the condition control:

1. Click **Edit** to open the **Edit Step/Ramp Values** dialogue:

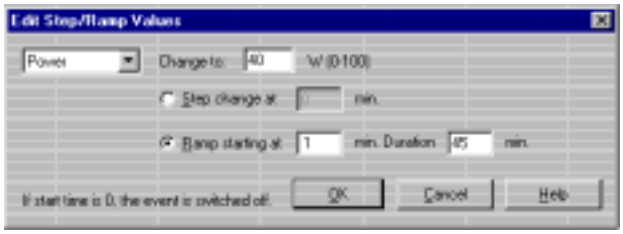

Figure 4-10. Edit Step/Ramp Values dialogue.

2. Open the menu to view and select the parameter that you want to step.

3. Edit the target value in the **Change to** field.

4. If a step change is required, select the **Step change at** button and enter the time at which the change is to occur.

   If a ramp change is required, select the **Ramp starting at** button, enter the time at which the change is to occur and the duration of time over which the change occurs.

5. Click on **OK** to complete the **Edit Step/Ramp Values** dialogue.

The change(s) you have entered are listed in the **Commands** field. If you want to remove any of the condition changes, select it in the **Commands** field and click on **Remove**.

Activating stepped pre-set changes for ALF*express*

You use the **Automatic condition control** to set a stepped change to the set conditions during the course of the run. This control allows you to preset a change in the:

- electrophoresis voltage
- current
- power
- temperature

The change can be executed as a step in value to the new value over a period of time. Each of the settings can have one step applied during the course of a run.

To set the condition control:

1. Click **Edit** to open the **Edit Step Values** dialogue:

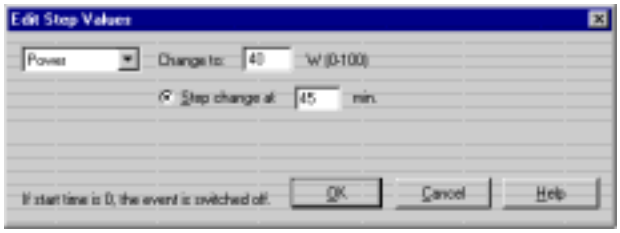

Figure 4-11. Edit Step Values dialogue.

2. Open the menu to view and select the parameter that you want to step.

3. Edit the target value in the **Change to** field.

4. If a step change is required, select the **Step change at** button and enter the time at which the change is to occur.

5. Click on **OK** to complete the **Edit Step Values** dialogue.

The change(s) you have entered are listed in the **Commands** field. If you want to remove any of the condition changes, select it in the **Commands** field and click on **Remove**.

4.4.3 Sample Information tab

This tab allows you to enter information about the samples, clones and the lanes in the gel. Information is entered in a tabular format, which allows standard editing procedures to be implemented so that you can quickly enter and edit information in the clone and lane sample information tables. Normally sample information is only retained in the result file but you can use the **Store table information in casebook file** option to retain sample information as part of the casebook file.

Adding sample information for the clones

1. From the **Casebook** dialogue, select the **Sample Information** tab. In **Samples Information**, select the **Clones** tab.

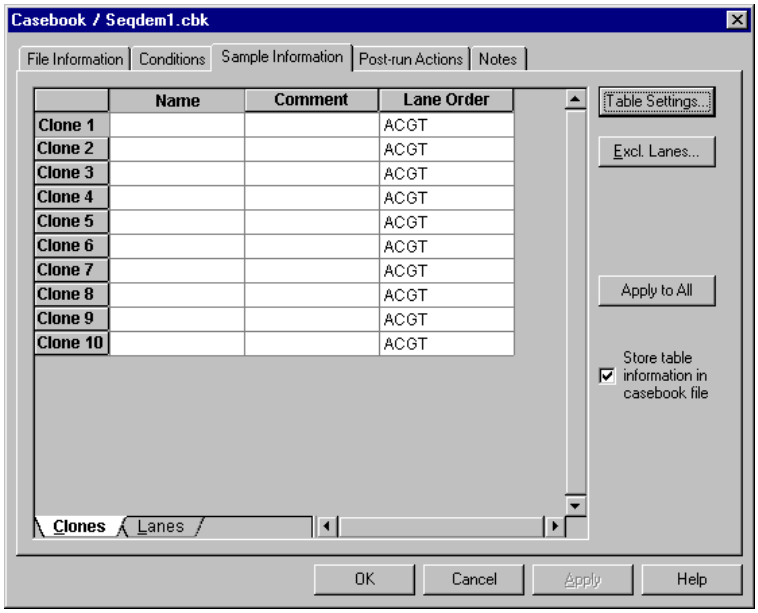

Figure 4-12. Casebook dialogue, Sample Information Clones tab.

2. Edit the information in the three default columns for sample **Name** and **Comment**. Several basic functions are available to add information, select the cells, and copy and paste information between cells:

Adding information

To fill in information about your lanes	Select a cell by clicking on it and type in the information.
To edit the **Lane Order**	Select the lane order cell. Click on the drop-down menu button and select the lane order required.

To move between cells	Use the left and right <cursor> keys to move around in the cell. Use the <Tab> key to jump to the cell in the next column or <Shift + Tab> to jump back to the adjacent cell in the previous column. Also use the up and down <cursor> keys to move within a column.
Selecting cells	
To select a single cell	Click on it or move to it by using the <tab> keys or up and down <cursor> keys.
To select a number of separate cells	Hold down the <Ctrl> key and click the cells.
To select a block of cells	Click on a cell at one corner of the block. Hold down the <Shift> key and click the cell located in the opposite corner of the block. Alternatively click on one cell and drag select the remainder.
To select a column of cells	Move the mouse pointer into the header at the top of the column. The pointer turns into a black arrow. Click on the left mouse button.
To select a row of cells	Move the mouse pointer into the margin at the left of the row. The pointer turns into a black arrow. Click the left mouse button.
To select the entire table	Click in the upper left corner of the table.

Moving and copying cell contents

To move the contents of one or more cells	Select the cell(s) to be moved and press the <Ctrl+X> keys. Select the cell located at the top left hand corner of the target position and press the <Ctrl+V> keys.
To copy the contents of one or more cells	Select the cells to be copied and press the <Ctrl+C> keys. Select the cell located at the top left hand corner of the target position and press the <Ctrl+V> keys.
To delete the contents of one or more cells	Select the cell(s) to be cleared and press the <Del>key or press the <Ctrl+X> keys.
To copy the contents of one cell to all the cells in the column	Select the cell to be copied. Click on **Apply to All**.

Table Settings...

3. Where appropriate, you can add up to another four columns to the table. To add further columns, click on the **Table Settings** button. The **Clones** dialogue opens.

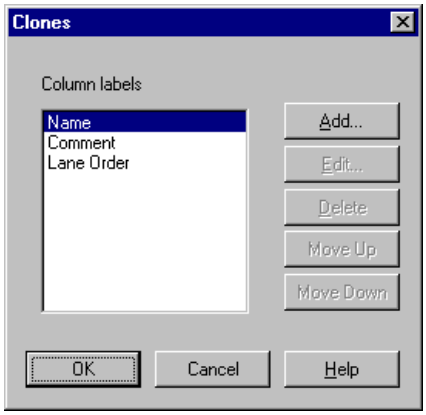

Figure 4-13. Edit Columns dialogue.

To add a column	Click on **Add**. Type the name of the column header in the dialogue and click on **OK**.
To edit a column header	Select the header and click on **Edit**. Type the changes in the dialogue and click on **OK**. Note that you cannot edit the column headers for the three default columns **Name**, **Comment** and **Lane Order**.
To delete a column	Select the column by clicking its name in the **Column labels** field. Click on **Delete** then click on **OK** to confirm the deletion. Note that you cannot delete the three default columns **Name**, **Comment** and **Lane Order**.
To change the order of added columns	Select the header to be moved and click on **Move Up** or **Move Down**.

4. Click on **OK** to view the changes to the columns in the table of sample information.

5. Add information to the new columns as appropriate.

6. Adjust the width and height of the cells to accommodate the sample information:

To adjust the width of cells	Move the mouse pointer into the top margin of the table and on to the cell boundary. The mouse pointer becomes a horizontal double arrow. Click and drag the cell margin.

To adjust the height of cells	Move the mouse pointer into the left hand margin of the table and on to the cell boundary. The mouse pointer becomes a vertical double arrow. Click and drag the cell margin.

7. Select **Store table information in casebook file** if it is useful to retain the sample information as part of the casebook file. If this option is not selected, the table contents are only saved in the result file for this run and you will have to rewrite the table the next time you use this casebook file.

Adding sample information for the lanes

The procedure for adding information on the lanes is similar to that for clones and the same cell editing procedures are used.

1. From **Sample Information**, select the **Lanes** tab.

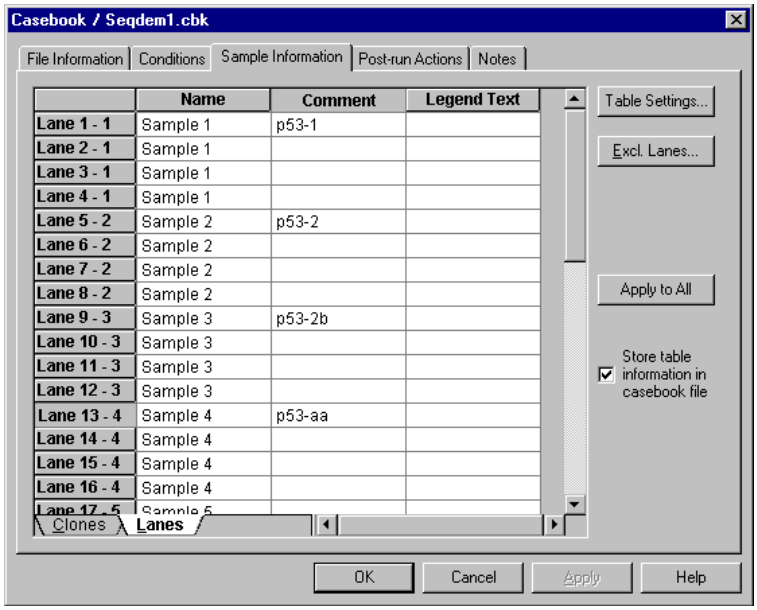

Figure 4-14. Casebook dialogue, Sample Information Lanes tab.

2. The default settings provide three columns, **Name**, **Column** and **Legend Text** and you can add up to four more columns. Click on the **Table Settings** button to add further columns as required and name their headers. Use the same procedure as you used for clones.

Note: You can rename all of the headers including the first three columns, but you cannot move or remove the first three columns.

3. Enter lane information into the cells of the table.

4. Select **Store table information in casebook file** if it would be useful to retain the sample information as part of the casebook. If this option is not selected, the table contents are only saved in the result file for this run and you will have to rewrite the table the next time you use this casebook file. If you have added or edited the sample information but not selected **Store table information in casebook file**, a warning is shown when you close the **Casebook** dialogue.

Excluding lanes

Lanes can be excluded from the data collection process.

Excl. Lanes...

1. To exclude lanes, either in the **Clones** or **Lanes** mode, click on the **Excl. Lanes** button to show the **Edit Lane Configuration** dialogue.

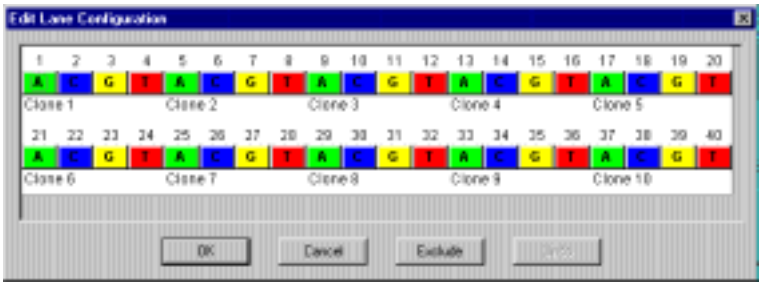

Figure 4-15. Edit Lane Configuration dialogue.

The four lanes for each clone are default colour coded according to the four bases. Any excluded lanes are represented by grey boxes labelled 'X'.

To exclude a lane	Select the lane and click on **Exclude** or double click on the lane. Note that lanes can only be excluded before the run is started.
To re-install a lane	Select the lane and click on **Undo** or double click on the lane.

2. Click on **OK**.
   Excluded lanes are marked with an asterisk (*****) in the **Lanes** view and are grey in the **Detector** view during a run.
   Clones are rearranged automatically in the **Lanes** view. When lanes are excluded, the remaining lanes are rearranged so that each clone is allocated the four lanes it requires.

   You can use the **Lanes** view to see which lanes are associated with each clone. For example, “Lane 2 - 1” means that lane 2 is included in clone 1. Excluded lanes are marked with “*****”, for example “Lane 3 - *****” means that lane 3 is excluded and is not included in any clone.The excluded lanes settings will not be stored in the casebook file.

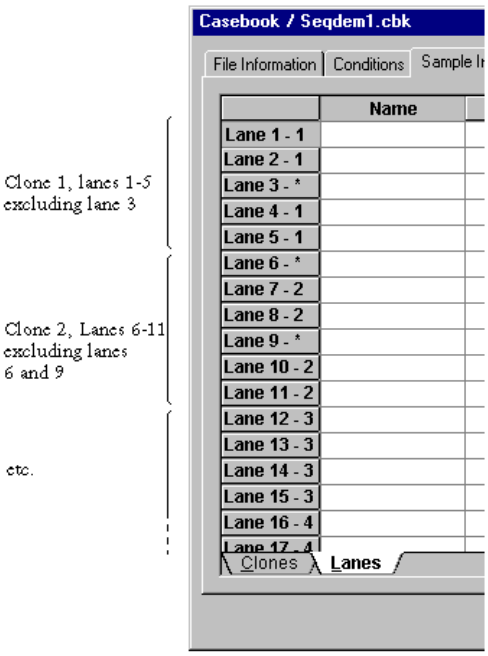

Figure 4-16. Casebook dialogue, Sample Information Lanes tab. Lanes that have been excluded are represented by an asterisk ‘*’, and the lanes belonging to each clone have been adjusted accordingly.

Warning:
The excluded lanes settings are lost if you close the casebook (**File:Close casebook**) or open another casebook. The excluded lanes settings are only stored in the result file

4.4.4 Post-run Actions tab

This tab allows you to specify activities that will proceed automatically when the run is completed. These include data processing, export, printing, launching tools and file backup.

The following tasks can be automated:

Process data	Process the raw data and produce a processed data file.
Export a sequence result	Convert the data into standard format and save it as a sequence file.
Print a report	Print a report containing selected information.
Produce a backup file	Safeguard the data by saving it in a secondary location.
Start tools	Open another software application when the processing is complete.

Note: All of these tasks, with exception to the production of a backup file, can also be accessed from ALFwin Sequence Analyser.

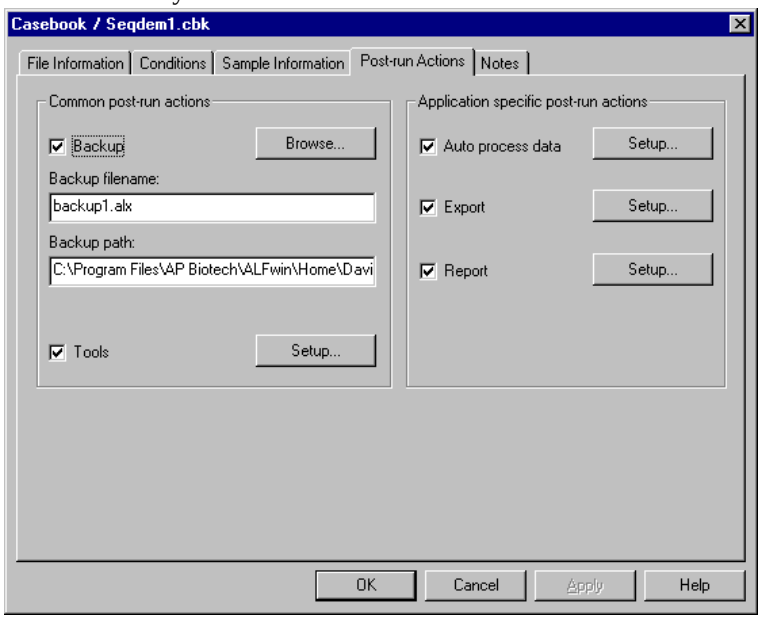

Figure 4-17. Casebook dialogue, Post-run Actions tab.

Each task can be customised to select the items to be included. For example, within **Print a Report**, you can specify the parts of the casebook that are printed. Tasks themselves can be enabled or disabled so that you can quickly alter the post-run actions required for a particular run. You can save the post-run action settings in the current casebook file before starting the run.

Automating the post-processing of raw data

It is possible to automatically process the raw data at the end of the run to generate the specific DNA sequence for each of the clones. Processed data is stored in the result file. This post-run action uses the default automatic settings for raw data processing from ALFwin Sequence Analyser. Raw data that has been processed using post-run actions can be viewed and analysed in the Sequence Analyser module, and you can process the data again with using a wider range of processing options (see Section 5.7).

1. From the **Post-run Actions** tab of the **Casebook** dialogue, select **Auto process data**.

2. Click on **Setup**. The **Process Setup** dialogue is shown.

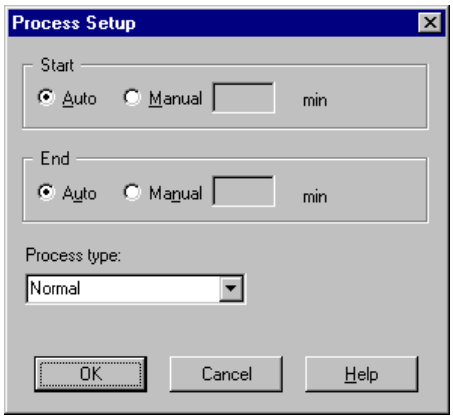

Figure 4-18. Process Setup dialogue.

3. Select the **Start** mode. The default is **Auto** which starts the processing after the highest peak, corresponding to the primer. To start the data processing at a fixed point along the time axis, select **Manual** and enter the time in the **min** field.

4. Select the **End** mode. Normally data processing is terminated when the peak quality is low, the signal level decreases or when the

resolution of the peaks is less (see Appendix C). This is the default **Auto** option. To stop data processing at a fixed point, select **Manual** and enter the stop time in the **min** field.

5. Select **Process type**.

Normal	Select this option when no heterozygote position is expected.
Heterozygote Low	Select this option when there is a low expectancy of finding ambiguity codes.
Heterozygote Medium	Select this option when there is a medium expectancy of finding ambiguity codes.
Heterozygote High	Select this option when there is a high expectancy of finding ambiguity codes.

6. Click on **OK** to complete setting up the automation of post-run data processing.

Automating the export sequence

You can export a sequence result in a format that is compliant with other applications. The options available are described below.

Note: It is not possible to obtain export files from raw data that has not been processed.

By default, the export function creates a file for each sequence (clone) but you can deselect any that are not required.

1. From the **Post-run Actions** tab in the **Casebook** dialogue select **Export**.

2. Click on **Setup**. The **Export Settings** dialogue is shown.

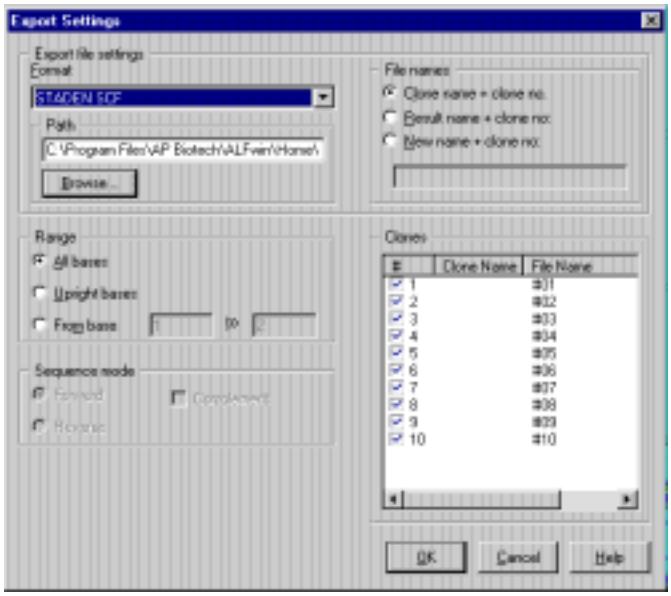

Figure 4-19. Export Settings dialogue.

3. Open the **Format** menu and select the export format you require:

GCG	The .seq format for GCG, the sequence analysis program from Wisconsin University.
STADEN SCF	The .scf format for the Staden sequence analysis package. SCF format includes curve data.
PC/GENE	The N prefixed format for PC/GENE, a sequence analysis program by IntelliGenetics from the Oxford Molecular group.
ASCII	ASCII .txt format that will load into many standard commercial applications.
EMBL	The .eml format for Bioresearch, the sequence analysis program by Fujitsu Ltd.
DNAsis	The .dna format of DNAsis, a sequence analysis program from Hitachi Software.

4. By default, all clones are selected. Click to remove the check mark from any clones in the **Clone** field that are not to be included in the export.

5. Select the **Range**:

All bases	All bases in the selected clones will be exported.
Upright bases	Only upright bases will be exported.
From base....to...	Selects the part of the sequence to be exported (for example, from base pair number 12 to base pair number 123).

6. Select the **File name(s)**:

Clone name + clone no.	The file will be named using the clone name + the clone number in the **Sample Information** tab.
Result name + clone no.	The file will be named using the result file name + the clone number.
New name + clone no.	Type a name. Files are saved using this name + the clone number.

7. Use the **Path** field to specify the location for the exported file. Type the path or click on **Browse** to select the required folder.

8. Click on **OK** to complete setting up the automation of export sequence.

Automating the printing of a report

The printed report consists of result curves and information from the casebook. You select the type of information that you require in the report.

1. From the **Post-run Actions** tab in the **Casebook** dialogue select **Report**.

2. Click on **Setup**. The **Report Settings** dialogue is shown.

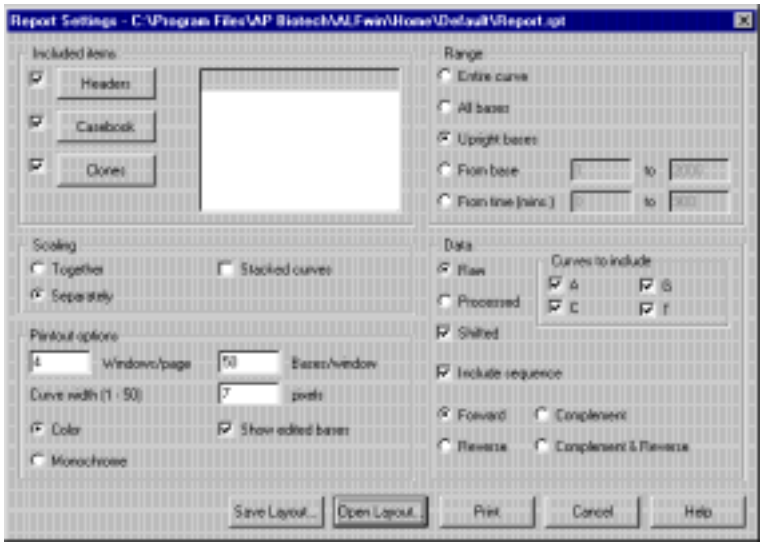

Figure 4-20. Report Settings dialogue.

3. You can use a report setting that was created and saved in a previous analysis (see step 10 below). To recall this setting, click on **Open Layout** and use the **Open** dialogue to select the path and the .rpt file name. Click on **OK** to load the settings. You can use the following procedures to edit the settings.

Note: The latest used and saved layout file in a casebook is automatically selected when you open the **Report Settings** dialogue.

4. Select the **Headers**, **Casebook** and **Groups** buttons in turn and select items to be included in the report from the lists shown. The white button must be checked to print the items selected in each group.

Headers This allows you to select the name, current user, current file name and current date in the present result file that are to be included in the header of the report.

Casebook This allows you to select items from the casebook and logbooks that are to be included in the report.

Groups This allows you to select the clones or groups to be included in the report.

Note: Groups must be selected to affect the following:

5. Select the **Range**:

Entire curve Print curve from the first to the last minute of the run.

All bases Print curves for the called bases.

Upright bases Print curves for the upright bases.

From base..to... Print curves for the range of bases you enter in the **From...to...** fields.

From time (mins.)..to... Print curves for the range of time you enter in the **From...to...** fields.

6. Select the **Scaling** to be applied to the print of the curves:

Together The amplitude scale for the curve containing the largest peak is used as the basis to scale the remaining curves. Thus, all peaks are correctly proportioned in relation to the largest visible peak.

Separately Each of the curves is scaled separately so that the displayed amplitude scale for each curve is adjusted to best fit the print space available.

Stacked curves Print the curves at four different levels in the curve window.

7. Select the type of **Data** to be included in the report:

Raw or Processed Print curves of raw or processed data.

Note: In order to print processed data curves, the raw data must first be processed as a post-run action.

Curve A, C, G and T Include the selected base curve(s).

Shifted Print the curves with the shift applied during processing to space the peaks at regular intervals. This only applies to processed data produced as a post-run action.

Include Sequence Include the base sequence in the curve print. This only applies to processed data produced as a post-run action.

Forward or Reverse Print the forward or reverse sequences/curves.

Complement Print the complementary of the forward sequences/curves.

Complement & Reverse Print the reverse of the complementary sequences/curves.

8. Select the **Printout options**:

Windows/page Enter the number of windows to be printed on each page.

Bases/window Enter the number of bases to be printed in each window.

Curve width Enter the width required for the printed curve line.

Colour or **Monochrome** Use the **colour** option when a colour printer is available for report printing. Use the **monochrome** option to produce black and white printouts which are useful if you wish to photocopy or fax the print. Different types of lines are used to differentiate between curves.

9. Click on **OK**.

10. If you want to save these settings for later use, click on **Save As** in the **Report Settings** dialogue and use the **Save Layout** dialogue to define the path and file name. Click on **Save**.

11. Click on **OK** to complete setting up the automation of print report.

Automating the production of a backup file

This function allows you to automate the creation of a backup of the result file that is generated at the end of a run. The backup copy should be saved in a drive different to that used for your result files, for example a server drive. Note that a diskette may not have sufficient capacity for the files created by a run.

1. From the **Post-run Actions** tab in the **Casebook** dialogue select **Backup**. By default, your backup folder will be selected if there was no other path stored in the casebook file from an earlier run.

2. Click on **Browse** and use the **Save As** dialogue to select the location and file name for the backup file.

4. Click on **OK** to complete setting up the automation of backup.

Automating the selection of tools

A tool is a shortcut to another program.The program you specify will be opened automatically when the run and post-run actions are completed.

1. From the **Post-run Actions** tab in the **Casebook** dialogue select **Tools**.

2. Click on **Setup**. The **Tools Setup** dialogue is shown.

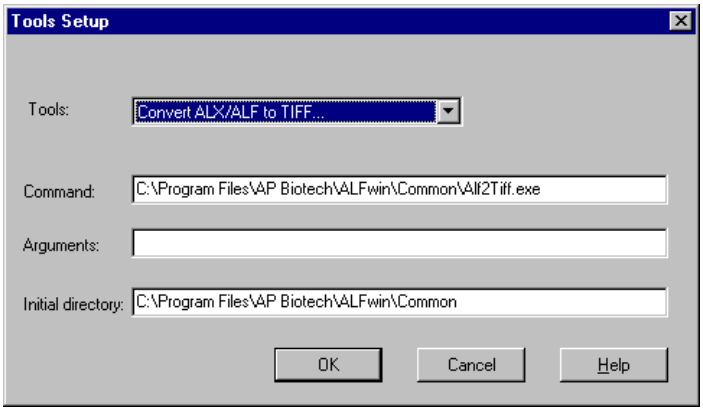

Figure 4-21. Tools Setup dialogue.

3. Open the **Tools** menu and select the tool required. There are two available tools supplied with ALFwin, **Convert ALX/ALF to TIFF** and **Decompress ALF file**. Please refer to Section 5.14.

   The path too the selected tool will be automatically given.

4. Enter, if required, parameters to start the program in the **Arguments** field. For example: “A file name”.

5. Click on **OK** to complete setting up the automation of tool selection.

4.4.5 Notes tab

This tab allows you to make your pre-run, run and analysis (post-run) notes. The casebook manages note taking, allowing access only to the appropriate type of note according to the stage reached in the experimental procedure. The **Notes** tab of the **Casebook** dialogue provides fields where you can write experimental notes. There are three fields in the **Notes** tab but only one is accessible at a time, according to the stage in the procedure:

Pre-run notes — Use this field to make notes before you start the run. The casebook is still accessible at this stage and you have the option to save pre-run notes in the casebook file. When you start the run, the **Pre-run notes** field is closed against any further entry.

Run notes — Use this field to make notes during the course of the run. When the run finishes, the **Run notes** field is closed against any further entry.

Analysis Notes — Use this field to make notes when using ALFwin Sequence Analyser.

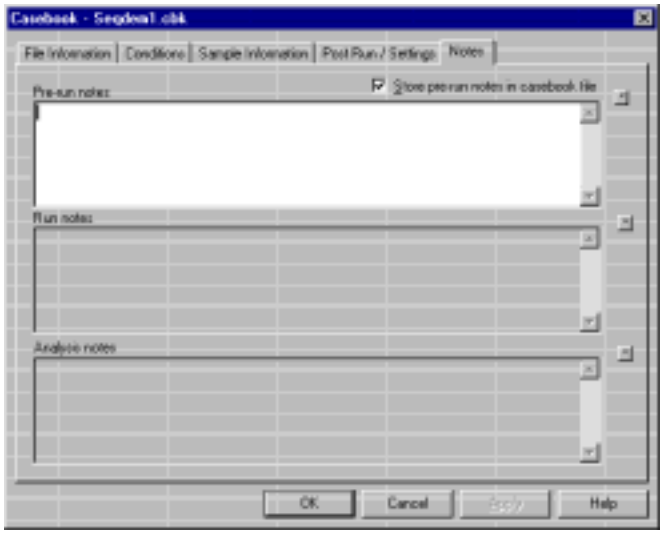

Figure 4-22. Casebook dialogue, Notes tab.

4.5 Performing a run

You can start a run once you have selected a casebook, made any modifications and saved the casebook file. A run can be divided into four main stages:

Preset	Preset is used before the start of a run to allow the gel and the buffer to achieve the correct temperature (see Section 4.5.1).
Starting the run	Starting the run allows ALFwin Control to automatically control and monitor a sequencing run using the connected instrument. When the run is started, any modifications made to the casebook during the run are saved in the result file only (see Section 4.5.2).
During the run	The instrument is controlled by the computer, according to parameters contained in the casebook. You can use the **Manual** window to observe the target parameters and compare them with their measured values. Adjustments can be made

manually as the run proceeds or you can pause the run to make adjustments. The **Logbook** is used to observe the progress of the run and automatically records any changes that are made. Changes include manual adjustments you make and step adjustments that have been programmed into the casebook. The **Curve** window is active throughout the course of the run so that you can observe the output of the light detectors and assess the quality of the raw curve data (see Section 4.6).

Ending the run

At the end of a run, ALFwin Control automatically saves the result file and implements any post-run procedures that you programmed in the casebook (see Section 4.7).

4.5.1 Preset and prepare the instrument

To preset the analyser:

1. Mount the gel cassette into the instrument and connect the thermostatic water circulator connectors.

2. Select **Run:Preset** or click on the **Preset** button. The run conditions are sent to the instrument. The preset order is logged in the **Logbook**. You can also see the set conditions in the **Manual** window on your screen. Black numbers are set values. Green numbers are actual values.

Note: You can set the temperature in the **Manual** window before you open or create the casebook. The temperature increases while you work with the casebook and saves you time later. Type the selected temperature and click on **Set.** The ALF*express* family instruments switch the heater on automatically but if you are using an ALF or ALFred instrument you must click **ON** at the **Heater** in the **Manual** window.

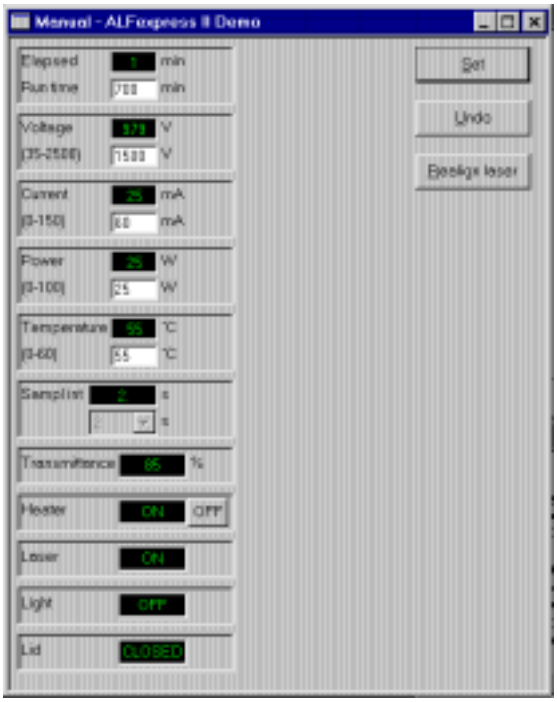

Figure 4-23. Manual window in ALF*express* II. Note that if you are using ALFexpress, the Manual window does not have the Realign Laser button and the Transmittance is expressed as the Laser value.

3. To see more of the run conditions in the **Manual** window, resize the window (see Appendix B "Short guide to Windows 95"), scroll the window contents using the scrollbars or change the font size using **View:Properties:Style:Font**.

4. To align/realign the laser using ALF*express* instruments, close and open the lid of the instrument to start the laser. If you are using an ALF or ALFred instrument, you must click **ON** at Laser in the **Manual** window.

5. Check the laser **Transmittance** in the **Manual** window. Transmittance is the value used in ALF*express* II to express the percentage of the laser light transmitted by the gel. Adjust the laser if necessary.

   If you are performing the run on an ALF*express* instrument, Transmittance is instead expressed as the **Laser value**, which is a measure of the accuracy of alignment of the laser beam.

6. Fill the upper and lower buffer reservoirs with the appropriate running buffers.

7. Wait for the selected temperature to be reached in the **Manual** window.

8. Prepare the instrument:

   remove the comb

   rinse the wells

   load the samples

   connect the electrodes.

9. Depending on which of the two ALF*express* instruments you are using, the laser is realigned by:

ALF*express* II	ALF*express*
clicking on the **Realign Laser** button in the **Manual** window.	gently pulling the cassette forward using the thermostatic circulator connections and rest the cassette back within one second. If the laser lamp goes out, restart the laser by closing the lid.

10. Close the lid of the instrument. The instrument is now ready.

4.5.2 Starting the run

To start the run:

1. Check that the laser and temperature lamps on the analyser are continuously illuminated.

2. Check temperature and the **Transmittance** value or **Laser value** for ALF*express* II or ALF*express*, respectively, in the **Manual** window.

3. Select **Run:Start** or click on the **Start** button. The **Start Run** dialogue is shown.

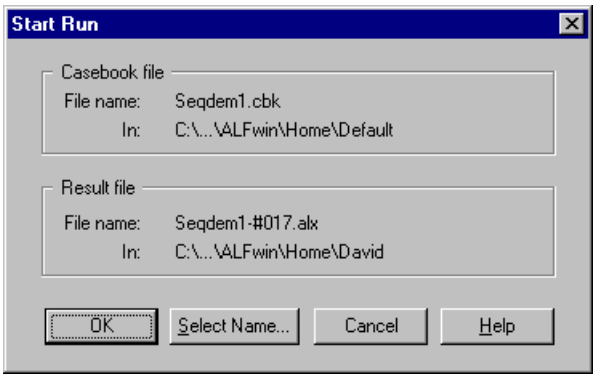

Figure 4-24. Start Run dialogue.

4. Check that the correct casebook is used and that the result file will be stored in the correct folder under the correct name. If you need to make changes, click on **Select Name** and type a new file name in the **Enter result file name** dialogue or select a new folder. Click on **Save** and then **OK**. Alternatively, you can click on **Cancel** and edit the casebook to change the file name and folder.

5. Click on **OK**. A message appears containing a time delay while the instrument checks that no residual daylight signal is saved from the detectors:

   "The sampling will start after 'x' seconds,
   please wait..."

   A green light in the title bar and in the Windows 95 taskbar confirms the run has started.

6. Wait until you can see curves in the **Curve** window to confirm that the electrophoresis is running.

4.6 Activities that can be performed during a run

There are various activities that you can perform during a run. These include:

- Pausing the run (see Section 4.6.1)
- Manually ending the run (see Section 4.6.2)
- Reloading the gel (see Section 4.6.3)

- Changing run conditions (see Section 4.6.4)
- Analysing running samples (see Section 4.6.5)
- Viewing curve amplitude information (see Section 4.6.6)
- Writing run notes in the casebook (see Section 4.6.7)
- Changing the screen layout (see Section 4.8)
- Starting a run on another instrument (see Section 4.6.8)
- Analysing other result files (see Chapter 5)

4.6.1 Pausing the run

You may need to pause the run to check or adjust the instrument. Do not pause for more than a couple of minutes otherwise the samples will diffuse. Opening the lid on the instrument pauses the run until the lid is closed again.

To pause the run:

1. Select **Run:Pause** or click on the **Pause** button. The run stops and the green lights turn red. Note that the laser and the heater are not affected by **Pause**.

2. To continue the run, select **Run:Continue** or click on the **Continue** button. A message counting down the time before the run resumes is displayed. the time delay allows the instrument to check that no residual daylight signal is saved from the detectors.

4.6.2 Manually ending the run

To manually end the run before the run time is completed:

1. Select **Run:End** or click on the **End** button. A dialogue asks you to confirm you want to end the run.

2. Click on **Yes**. The run ends and a dialogue asks if you want to perform post-run actions.

3. Select **Yes** or **No**.

4.6.3 Reloading the gel

You use the **Reload** command to end a current run and start a new run using the same gel. **Reload** allows access to the **Casebook** dialogue so that you can edit the casebook before re-starting the run. A reload is automatically documented in the logbook.

1. Select **Run:Reload**. A warning dialogue is displayed asking you to confirm your action. Click on **OK** to proceed.

   Post-run actions are executed and the **Samples/Standards** tab of the **Casebook** dialogue is shown.

2. Use the **Casebook** dialogue to edit the pre-run notes and samples/ standards as appropriate.

3. Click on **OK** to close the casebook.

4. The **Start Run** dialogue is shown. Check the result file name and click on **OK**.

5. Reloading is noted in the logbook in the form “Reload #x” where “x” is incremented for each reload.

4.6.4 Changing the run conditions

Warning:
Do not exit ALFwin Control or shut off the computer during the run otherwise the data from the run will not be saved. If you try to exit ALFwin Control, you are warned that you cannot do so during a run. Press **OK** to close the dialogue.

To change set values in the **Manual** window:

1. Type or select the new values in the lower box (black characters) of the conditions to be changed.

2. If you want to cancel these new settings, click on **Undo**. (This command can only be used before you click on **Set**.)

3. Click on **Set**. New values are sent to the instrument and can be seen in the **Logbook** window. Actual values (green characters) will change after a while.

Figure 4-25. Manual window in ALF*express* II. Note that if you are using ALFexpress, the Manual window does not have the Realign Laser button and the Transmittance is expressed as the Laser value.

4.6.5 Analysing running samples

You can analyse the present run before it is finished.
Before you open ALFwin Sequence Analyser, save the part of the run that is ready:

1. Select **Run:Pre-result**. The **Enter name for pre-result** dialogue is shown.

2. Type a name for the pre-result file. Choose a different name from that of the present result file.

3. Click on **Save**.

Note: No post-run actions will be performed at this stage.

4. Start ALFwin Sequence Analyser to check and analyse the pre-result.

Locate and select ALFwin Software from the Windows 95 Start menu options. In the **ALFwin Administrator** dialogue select your user name and then **Sequence Analyser 2.00** from the **Available applications** list. Click on **Start** and ALFwin Sequence Analyser starts (see Chapter 5).

4.6.6 Viewing curve amplitude information

To examine the amplitude of the curves:

1. Click on the **Curve** window to activate it.

2. Select **View:Signal Levels** or press the (<Ctrl+Shift+S>) keys. The maximum and minimum amplitudes and the difference in amplitude for each curve is shown in a table.

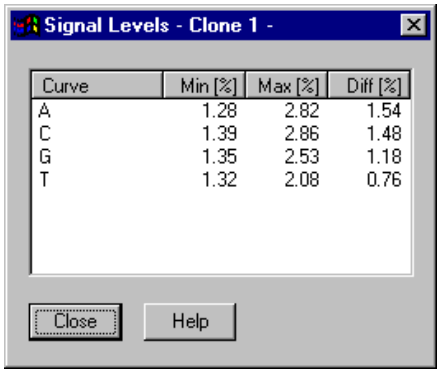

Figure 4-26. Signal levels dialogue.

The table relates to the parts of the curves and the current curve group shown in the curve window and the amplitude values are updated if you scroll in the curve window or change curve group.

The difference column shows the difference between the minimum and maximum signal level of the curve. Differences in the order of 1% are normal.
The **%** unit refers to the percentage of the maximum possible amplitude.

4.6.7 Writing run notes in the casebook

You can write **Run notes** in the casebook. Select **Edit:Casebook** and select the **Notes** tab. Changes and additions can not be made in this field after the run is finished.

4.6.8 Starting a run on another instrument

You can start a run on another instrument with ALFwin Control. This requires that you open a second copy of ALFwin Control (see Section 4.1.3)

4.7 After the run is finished

The run stops automatically when the run time is out. The selected post-run actions are then performed. The lights in the title bar and the taskbar turn red (post-run action) and then grey (not running). Run data are saved in the result file. You can read the casebook but not make any further changes.

To start the next run, open or create a casebook file (**File:Open Casebook** or **File:New Casebook**). To use the same casebook file as the previous run, you can select it from the list at the bottom of the **File** menu.

4.8 Changing the screen layout

This section describes how to configure the layout of your screen.

4.8.1 Arranging open windows

You can decide how to arrange all the open windows using commands in the **Window** menu.

To arrange the windows on the screen, select:

Window:Cascade	To position all open windows on top of each other.
Window:Tile as Default	To position the windows in the default tiled configuration.

Refer to Appendix B “Short guide to Windows 95” for a description of how you move and resize the **Curve**, **Manual** and **Logbook** windows.

4.8.2 Saving window positions on exit

To save the present positions of the windows on the screen and also the font settings in the **Manual** and **Logbook** windows for the next time you open ALFwin Control:

1. Position the windows as you want them on the screen.

2. Select **Window:Save Window Positions on Exit**. The windows will be in these positions the next time you open ALFwin Control. Operation of this command does not affect the default tiled positions that can still be recalled using the **Window:Tile as Default** option.

4.8.3 Hiding and showing the toolbar and status bar

Command buttons on the Toolbar help you speed up your work. The status bar gives you information about what is currently happening.

You can toggle these bars on and off by selecting **View:Toolbar** or **View:Status Bar**. Toolbar buttons cannot be changed.

4.8.4 Changing the look of the curves

This section explains how you can change the way the curves are shown in the **Curve** window. The run must be started before these commands can be used.

Show the Curve or Detector view

Normally the result from the run is shown as a series of curves that plot the signal level against time (**Curve** view). You can also choose to view the momentary signal level for all lanes at the same time (**Detector** view). These views are automatically updated after each signal sample.

To change from **Curve** view to **Detector** view:

1. Click on the **Curve** window to make sure that it is activated.

2. Select **View:Detector View** or click on the **Detector View** button. The detector view is shown in the **Curve** window. To return to curve view, select **View:Curve View** or click on the **Curve View** button.

The signal levels are normally very low compared to the dynamic range (maximum detector values). You can zoom in on the signals by entering a lower value for maximum signal level.

1. Click on the **Curve** window.

2. Select **View:Properties**.

3. Select the **Settings** tab.

4. Enter a value in the **Max Signal Level** field.

5. Click on **Apply** to effect the change.

6. Click on **OK** when the scale is satisfactory.

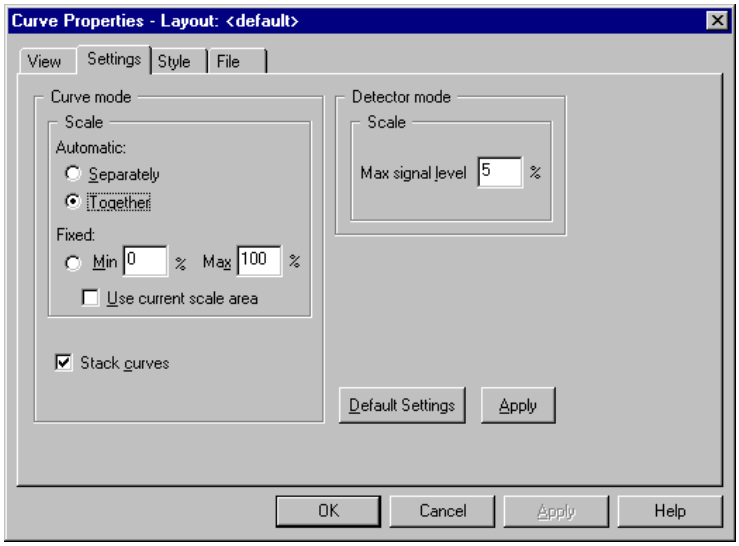

Figure 4-27. Curve Properties dialogue, Settings tab.

4.8.5 Showing stacked or overlapping curves

By default the curves are displayed stacked above each other in the **Curve** window. To view overlapping curves:

1. Click on the **Curve** window to make sure that it is activated.

2. Select the active (checked) **View:Stack Curves** to deactivate the function or click on the active **Stacked Curves** button. Overlapping curves are shown.

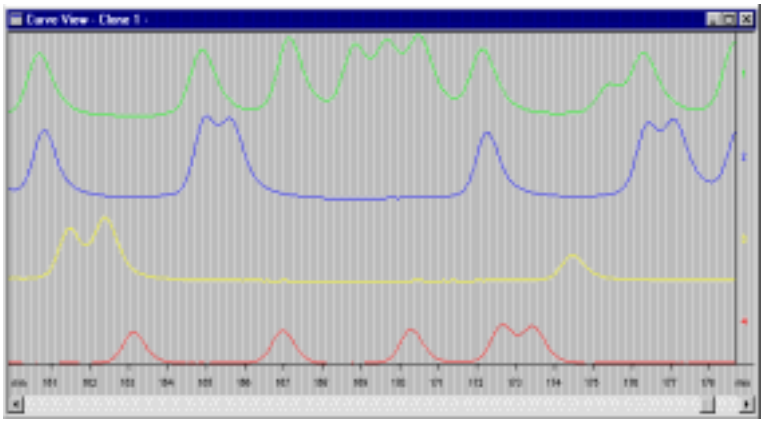

Figure 4-28. Curve view displaying stacked curves.

4.8.6 Using the Zoom function with curves

You can alter the magnification of the curves view in the **Curves** window.

Changing magnification with the zoom commands:

Command	*Effect*
View:Zoom In <F7> key	Successively increases the magnification.
<Shift+F7>	Increases the magnification in small steps.
View:Zoom Out <F8> key	Successively decreases the magnification.
<Shift+F8>	Decreases the magnification in small steps.

Increasing the magnification using the mouse

To magnify a specific portion of the window using the mouse.

1. Position the mouse pointer on the left side of the area you want to magnify.

2. Press and hold the left mouse button and drag the box to encompass the time interval to be magnified.

3. Release the mouse button.

Resetting the magnification

To reset the magnification factor to the default level, select **View:Reset Zoom** or press the <F9> key.

4.8.7 Selecting the group to be shown.

To choose the group of lanes to be shown in the **Curve** window, open the list of Groups in the toolbar and select the group required. Alternatively, double-click in the **Curve** window to show the next.

By default, **Group 1** displays the curves for all of the lanes and **Group 2** displays the lanes containing external standards. The remaining default groups **+Group 1** to **+Group 5** show groups of eight curves together.

The next section describes how you can create your own lane groups.

4.8.8 Creating your own lane grouping

To create your own lane group:

1. Click on the **Curve** window to make sure that it is activated.

2. Select **View:Properties**. The **Curve Properties** dialogue is shown.

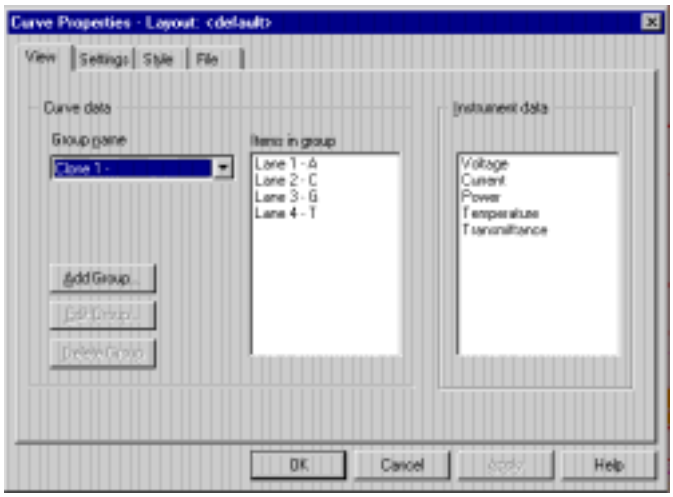

Figure 4-29. Curve Properties dialogue, View tab for ALF*express* II. If you are using ALFexpress, Transmittance is instead expressed as Laser value.

The **Group name** menu lists the names of existing groups. The **Items in group** field lists the lanes included in the group currently selected in the **Group name** list. The contents of the present group are highlighted.

Any instrument data included with the curves is highlighted in the **Instrument data** field.

3. Click on **Add Group**. The **+Group** dialogue opens.

4. In the **Group name** field, enter a name for the new group.

5. Select the lanes to be included in the group. Use <Ctrl + click> to add items spread out in the lists. Use <Shift + click> to add items that are next to each other.

6. Click on **OK**. The new group is added to the list of **Group names**. The name you entered is always added after "+Group #-".

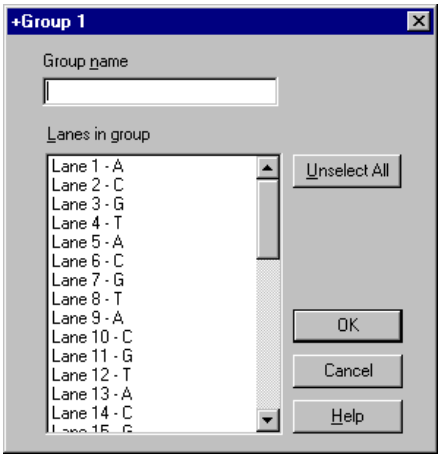

Figure 4-30. Group dialogue.

7. If required, select the **Instrument data** (one or more) to be displayed in all the curve windows. To deselect all data, <Ctrl+click> on the selected data.

8. Click on **OK** to exit the **Curve Properties** dialogue.

4.8.9 Editing a group

You can edit groups that you have added. These groups are marked with a "+" before the name.

To edit a group:

1. Click on the **Curve** window to make sure that it is activated.
2. Select **View:Properties**. The **Curve Properties** dialogue is shown.
3. Select the Group to be edited from list of **Group names**.
4. Click on **Edit group**. A dialogue is shown.
5. Change the name of the Group or lanes to be included in the group. The lanes already included in the group are highlighted.
6. Click on **OK**. The changes are made to the selected group.

4.8.10 Deleting a group

You can delete groups that you have added. These groups are marked with a "+" before the name.

To delete a group:

1. Click on the **Curve** window to make sure that it is activated.
2. Select **View:Properties**. The **Curve Properties** dialogue is shown.
3. Select the Group to be deleted from list of **Group names**.
4. Click on **Delete group**. A dialogue asks if you are sure you want to delete the group.
5. Click on **OK**. The selected group is deleted from the list of Group names.
6. Click on **OK** to close the dialogue.

4.8.11 Changing the Y-axis scaling

You can use menu commands to select the type of scaling for the currently selected clone **Curve** window:

Command	*Effect*
View:Scale:Separately	This automatically scales each of the curves separately so that the displayed amplitude scale for each curve is adjusted to best fit the window displayed. This may be useful in discerning the peaks in a curve that has a relatively low detection signal during sequencing. Note that selection of separate scales can be misleading when, for example, trying to establish if a peak is a true peak or background noise.
View:Scale:Together	This automatically maximizes the size of the largest peak visible in the current view of the **Curves** window. The amplitude scale for the curve containing this maximized peak, in the current view, is used as the basis to scale the remaining curves. Thus, all peaks are correctly proportioned in relation to the largest visible peak. This allows you to look at the relative signal detection sensitivity of the curves for the area of the result that you are viewing in the **Curve** view.
View:Scale:Fixed	This displays the portion of the curve that corresponds to minimum and maximum amplitude limits that you have defined in the **Curve Properties** dialogue (see below).

To change the scale for the **Curve** window:

1. Click on the **Curve** window.

2. Select **View:Properties** and click on the **Settings** tab in the **Curve Properties** dialogue.

Note: If you change the properties, the changes will affect all curve groups.

3. Select the relevant **Y-axis Scale**, either **Separately** or **Together** (see above), or **Fixed**. If you select **Fixed**, you must enter a minimum and maximum amplitude value, e.g. an entered range of 0% to 50% means that the portion of the curves with amplitude between 0% and 50% of the possible range is shown.

4. If you want the scale to be fixed according to the current scale area of the automatically calculated scales, i.e. as calculated for **Separately** or **Together**, check the **Use current scale area** box. Whenever you select **Fixed**, you can scroll along the result and view the peaks at their real height relative to the maximum amplitude for the whole run.

5. To implement the changes without closing the dialogue, click on **Apply**. To close the dialogue and effect the changes, click on **OK**.

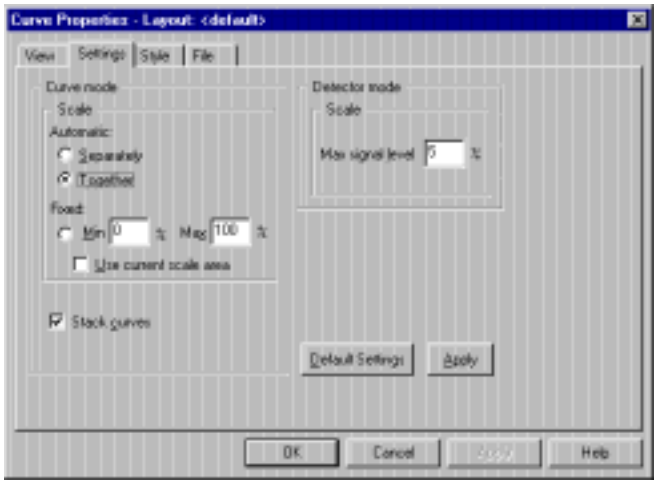

Figure 4-31. Curve Properties dialogue, Settings tab.

4.8.12 Changing fonts and the colours in the Curves window

You can change the colour of the curves and the background. You can also change the font for the X-axis and legends.

To change colours:

1. Select the **Curve** window.

2. Select **View:Properties** and click on the **Style** tab in the **Curve Properties** dialogue.

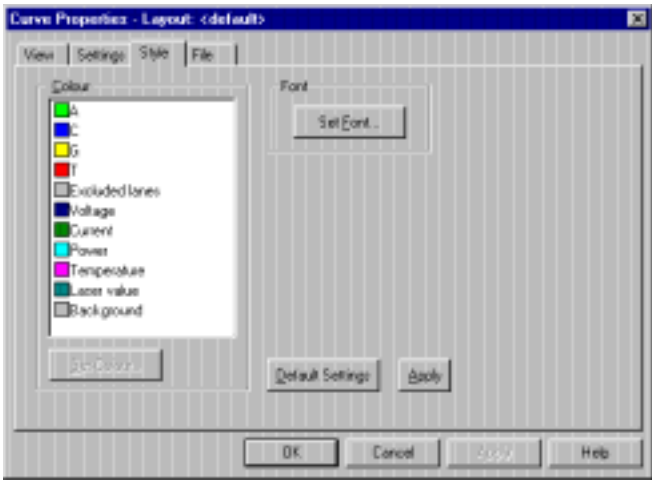

Figure 4-32. Curve Properties dialogue, Style tab.

3. To change colour, select the appropriate item under the **Colour** list and click on the **Set Colour** button. The **Colour** dialogue is shown.

4. Select a colour. To make your own colour, first define the colour and then click on **Add Custom Colour**.

5. Click on **OK**.

6. To implement the changes without closing the dialogue, click on **Apply**. To close the dialogue and effect the changes, click on **OK**.

Note: Only the solid colours can be used for curves, printed and exported to clipboard.

To change the scale and legends font:

1. Click on the **Set Font** button. The **Font** dialogue is shown.

2. Select a **Font**, **Font style** and **Size**.

3. Click on **OK**.

4. To effect the changes without closing the dialogue, click on **Apply**. To close the dialogue and effect the changes, click on **OK**.

If you want to restore the default font and colour settings, click on the **Default Settings** button.

4.8.13 Saving or opening a layout for the Curve window

You can save a **Curve** window layout for later use. All properties of the **Curve** window defined in the properties tabs are saved. If you do not save these, the default layout will be applied when you next use ALFwin.

To save a layout or open a saved layout:

1. Select the **Curve** window.

2. Select **View:Properties** and click on the **File** tab in the **Curve Properties** dialogue.

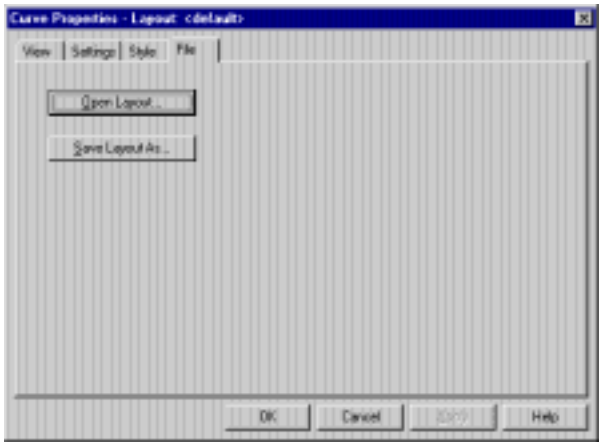

Figure 4-33. Curve Properties Layout dialogue, File tab.

3. Select a button:

Open Layout	The **Open** dialogue is shown. Use this dialogue to open a saved layout and apply it.
Save Layout As	Opens the **Save As** dialogue which allows you to save the layout as a **.lyt** file which you can use again later. The last used layout file will be applied by default the next time ALFwin Control is started by the same user. Use the dialogue to locate and name the layout file.

5. Click on **OK**.

4.8.14 Changing the appearance of the Manual window

You can select the parameters that are displayed in the **Manual** window and you can choose the fonts that are used. The settings are saved for the current user and are used the next time that ALFwin Control is started.

Changing the contents of the Manual window

Select the parameters to be displayed in the **Manual** window. All parameters are selected as default.

To select parameters:

1. Click on the **Manual** window to make sure that it is activated.

2. Select **View:Properties**. The **Manual View Properties** dialogue is shown. Select the **Window Contents** tab.

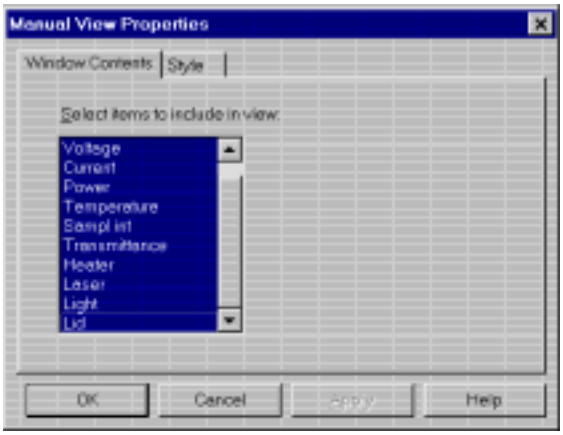

Figure 4-34. Manual View Properties dialogue, Window Contents tab for ALFexpress II. Note that if you are using ALFexpress, Transmittance is expressed as the Laser value.

3. Select the items in the list to be displayed in the **Manual** window. Click to activate or de-activate each item.

4. Click on **OK**. The selected items are displayed in the **Manual** Window.

4.8.15 Changing the font in the Manual window

Changing the font in the **Manual** window adjusts the size of the fields in the window. The field for each parameter changes size according to font size.

To change the font:

1. Click on the **Manual** window to make sure that it is activated.

2. Select **View:Properties**. The **Manual View Properties** dialogue is shown.

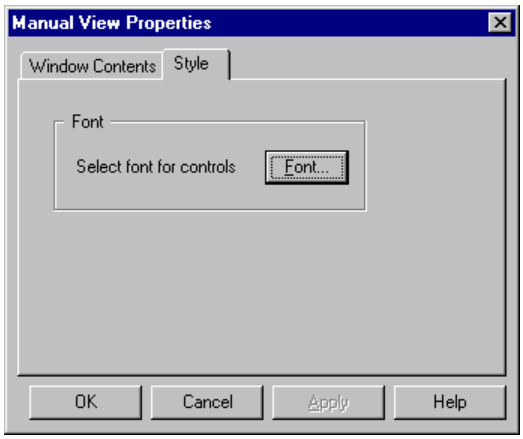

Figure 4-35. Manual View Properties dialogue, Style tab.

3. Select the **Style** tab.

4. Click on the **Font** button and use **t**he **Font** dialogue to select the **Font**, **Font style** and **Size**.

6. Click on **OK**.

7. Click on **Apply** to view the results without closing the dialogue or click on **OK** to change the font and close the dialogue.

4.8.16 Changing the font in the Logbook window

You can select the font to be used in the **Logbook** window. The font settings are saved for the current user and are used when ALFwin Control is next started.

To change font:

1. Click on the **Logbook** window to make sure that it is activated.

2. Select **View:Properties**. The **Logbook View Properties** dialogue is shown.

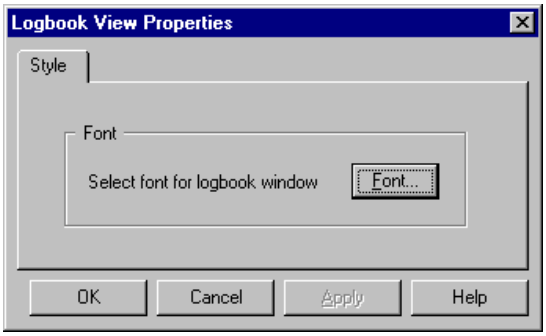

Figure 4-36. Logbook View Properties dialogue, Style tab.

3. Click on **Set Font**. The **Font** dialogue is shown.

4. Select the **Font**, **Font Style** and **Size**.

5. Click on **OK**.

6. Click on **Apply** if you want to view the results without closing the dialogue.

7. Click on **OK**. The font is changed.

4.9 Exiting ALFwin Control

To exit ALFwin Control when you are not performing a run, i.e. when the indicator light in the Windows 95 taskbar is grey:

1. Check that no samples are running. The lights should be grey and the last line in the Logbook window should begin "Run ready at...., followed by three more lines confirming that the default values have been sent to the instrument. The instrument is ready for a new run.

   If you try to exit ALFwin Control while a run is in progress, you are warned that you cannot quit during a run. Press **OK** and the run continues normally.

2. Select **File:Exit**. If Control was entered from Sequence Analyser, you are returned to Sequence Analyser. Otherwise, ALFwin Control is closed.

5 ALFwin Sequence Analyser

After you have obtained the run data in ALFwin Control, the results can be evaluated in ALFwin Sequence Analyser. ALFwin Sequence Analyser should be used to achieve four main goals:

- To extract a nucleotide sequence from the raw data using the automated data processing algorithms
- To present the data on paper by printing either direct from screen or by use of the powerful Report function.
- To export the data in appropriate formats for direct use in other software applications
- To check that the run was successful
- To analyse the sequence data and make judged improvements to the quality of the data

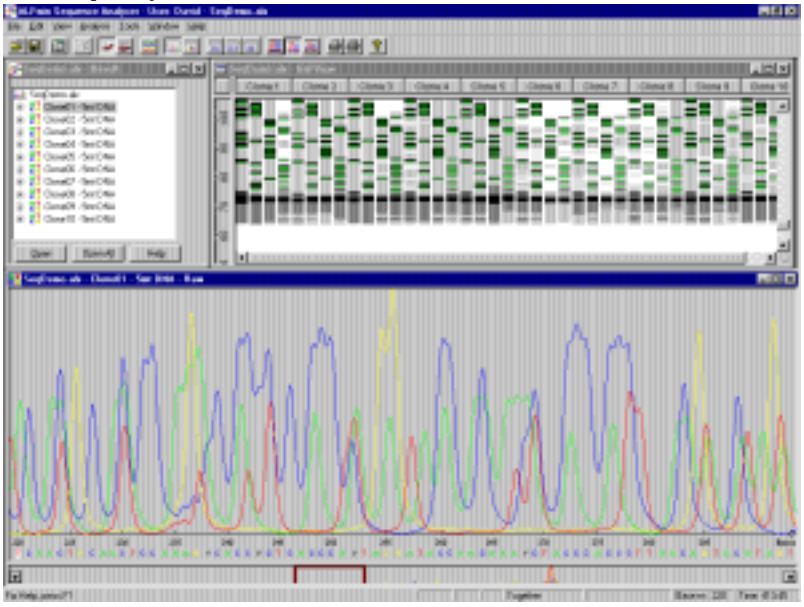

Figure 5-1. Typical screen display in ALFwin Sequence Analyser.

In order to achieve these goals, ALFwin Sequence Analyser has been designed with a wide range of powerful tools, including:

Data processing options — ALFwin Sequence Analyser contains many processing options, for example altering the stringency of the data processing based on the degree of heterozygocity in the

	samples; automatically or manually defining the start times of the run; edit shift in raw data curves to account for heterogeneous running speed in the gel.
Editing facilities	Powerful editing facilities are provided that enable you to directly replace, insert or delete bases in the sequence.
Sequence alignment	Sequence alignment is a separate module within ALFwin Sequence Analyser that allows you to align sequences within the same or different result files. This enables you to view base-specific differences between aligned sequences and directly edit the sequence data accordingly. Sequence Alignment is presented in Chapter 6.
Presentation tools	In order to properly analyse the data, ALFwin Sequence Analyser has very flexible data presentation options both in curve view and also in gel (fluorogram view). You are able to view one or several sets of clone data simultaneously, to zoom in on selected areas of a run and to move between views for raw data and processed data. All data is colour coded and presentation preferences easily changed for maximum comfort. Much of the editing work is performed in the curves view.

All of the actions that you perform in ALFwin Sequence Analyser are logged so that you can always look back on what you have done. Moreover, you can print out a wide selection of the results of your evaluation, either direct from screen or in a report.

This chapter is divided into the following sections:

5.1 Starting ALFwin Sequence Analyser
5.2 Introducing the ALFwin Sequence Analyser interface
5.3 Opening a result file
5.4 Presenting results in the Result window
5.5 Presenting curves in the Curve window
5.6 Presenting the run in the Gel View window
5.7 Processing run data
5.8 Editing sequence bases
5.9 Viewing casebook information and adding evaluation notes
5.10 Saving the result file
5.11 Exporting data
5.12 Printing a report
5.13 Printing Curve and Gel Views
5.14 Using tools
5.15 Exiting ALFwin Sequence Analyser.

5.1 Starting ALFwin Sequence Analyser

5.1.1 Starting ALFwin Sequence Analyser

ALFwin Sequence Analyser is started from the ALFwin - Administrator dialogue.

1. Locate and select ALFwin from the Windows 95 Start button menu options. The **ALFwin - Administrator** dialogue is shown.

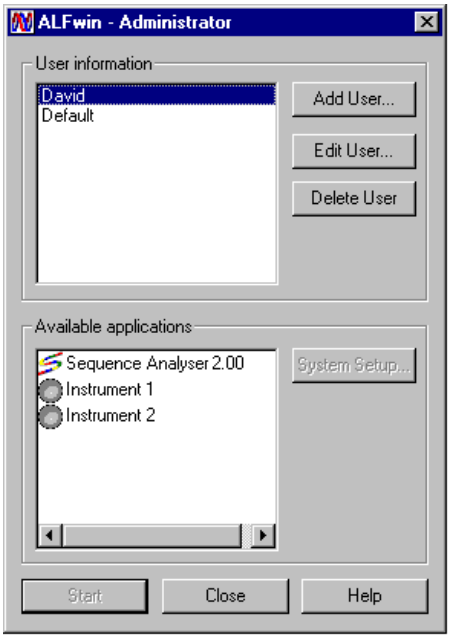

Figure 5-2. ALFwin Administrator dialogue.

2. In the **ALFwin - Administrator** dialogue, select your user name or the name **Default** and then double click on **ALFwin Sequence Analyser 2.00** under **Available applications**.

   The ALFwin Sequence Analyser module is started. For more information on the Administrator module, see Chapter 2.

3. When ALFwin Sequence Analyser starts, the splash screen is displayed for a few moments and is then replaced by the ALFwin Sequence Analyser screen. The **Tip of the Day** dialogue is superimposed on the screen.

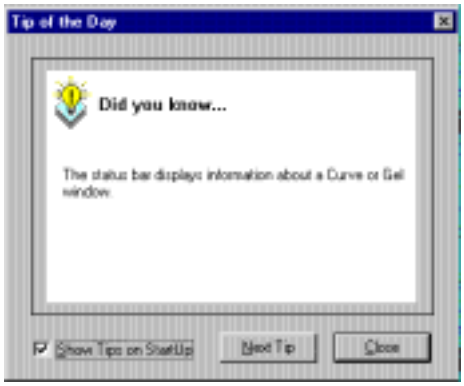
Tip of the Day
Did you know...
The status bar displays information about a Curve or Gel window.
Show Tips on StartUp
Next Tip
Close

Figure 5-3. Tip of the Day dialogue.

You can read tips about using the program. To view more tips, click on the **Next Tip** button. If you do not want to see the dialogue at each startup, uncheck the **Show Tips on StartUp** box.
You can open the **Tip of the Day** dialogue from the program by selecting **Help:Tip of the Day**.
Click on **Close** to leave this dialogue.

5.2 Introducing the ALFwin Sequence Analyser interface

5.2.1 Windows

Three main windows are used to analyse results contained in a result file:

The **Result** window	Clone and lane information is shown in a **Result** window for each opened result file.
The **Curve View** window	This provides a graphical view of run data for a selected clone for raw data or processed data.
The **Gel** window	This shows the run data for the whole run as a fluorogram.

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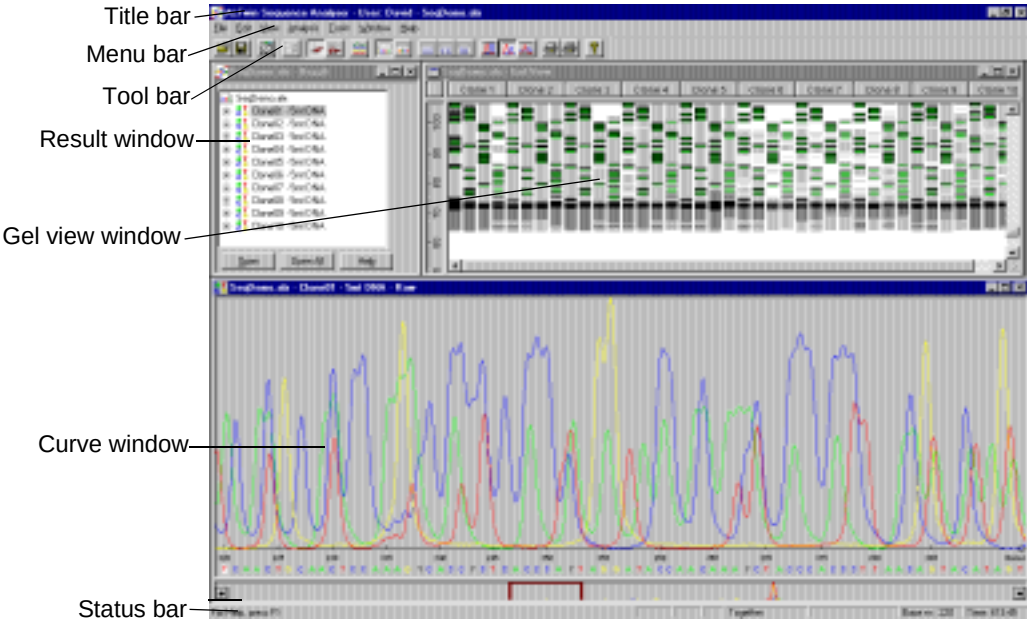


Figure 5-4. ALFwin Sequence Analyser screen with the Curve window and Gel View window open displaying results for the result file open in the Result window. Note that you can maximize the display of any window or change the size of the windows by dragging the window borders.

5.2.2 Bars

The following bars are displayed on the screen:

Title bar	This shows information about the name of the open result file and the name of the selected user.
Status bar	This gives you detailed information about, for example, the position of the mouse pointer and current mode selected on the screen. You can hide the status bar by deselecting (unchecking) the active **View:Status Bar** command.
Menu bar	Click on any menu option to view and select the specific commands.

Toolbar	The toolbar buttons allow you to quickly implement the most commonly used commands. The range of active buttons is dependent upon the currently active window in the workspace. Unavailable buttons are greyed out or not shown. Use the mouse pointer to get a description of each button. You can hide the toolbar by deselecting (unchecking) the active **View:Toolbar** command.

5.3 Opening a result file

This section describes how to open one or several result files after a sequencing run and select to view the data in different presentation modes.

ALFwin Sequence Analyser uses the current .alx result file format but can also read data in the earlier .alf format. If the .alf data have been saved in compressed format, it is necessary to decompress the files before they are used by ALFwin Sequence Analyser. Please refer to Section 5.14.2 which describes this procedure.

5.3.1 Contents of a result file

A result file contains the following:

Raw data	Raw data curves for each of the clones included in the run.
Casebook	This contains all documentation that you included in the casebook in the Control module in addition to the logbook and evaluation log information. This information cannot be changed although new notes regarding the evaluation process and sample information can be added in addition to the automatic generation of an evaluation log.
Processed data	The processed data curves for each of the clones included in the run sequence are included if they were generated by post-run action or added here after processing has been performed and the result saved.
Sequence	The bases called by the base calling algorithm.

5.3.2 To open a result file

To open a result file:

1. Select **File:Open** or click on the **Open** button. The **Open** dialogue is shown. Alternatively, you can directly open one of the eight most recently saved files from the list in the **File** menu.

2. In the **Open** dialogue, select the drive and folder containing the appropriate .alx or .alf result file. Select the file and click on **Open**. You can open several files by pressing the <Ctrl> key while you select the files. In this case all the files selected will be opened when you select **Open**.

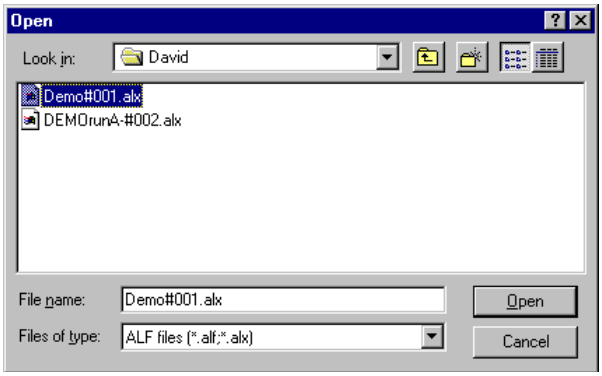


Figure 5-5. Open dialogue for selecting result files.

The **Result** window (see Figure 5.6) is displayed in the ALFwin Sequence Analyser workspace.

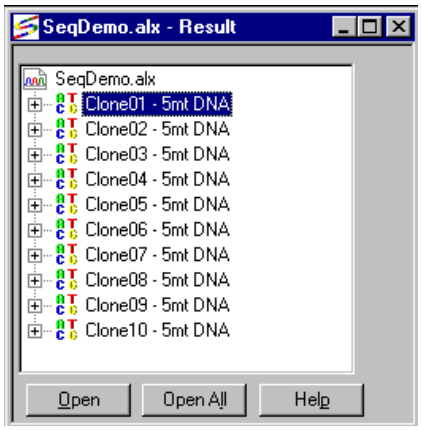


Figure 5-6. Result window.

3. Repeat step 1 if you want to open more result files.

   Note: All opened result files are visible on the ALFwin Sequence Analyser desktop. This means that you can open other windows, for example **Curve** windows (see Section 5.4.1), for any of the clones in the result files.

4. Switch between open windows in the **Window** menu.

   Note: In ALFwin Sequence Analyser, you can open an .flx file but you can not save results in .flx format.

5.4 Presenting results in the Result window

The **Result** window contains a tree structure of all the clones contained in the opened result file. From this window it is possible to open the curves for each clone (see Section 5.4.1) and to process the data (see Section 5.7).

5.4.1 Clone icons

In the **Result** window, each clone is represented by an icon to indicate whether it is raw data or if the data have been processed:

Raw data - the run data received from the instrument.

Processed data - these data have been processed i.e. the bases in the sequence have been called by the base-calling algorithm

Edited processed data - these data have been processed and then manually edited.

These icons are displayed in other windows with respect to the clone data being presented.

5.4.2 Viewing the lane information

The sequencing lanes for a clone can be viewed by clicking on the **+** symbol beside the clone, or conversely hidden from view by clicking on the corresponding **-** symbol. Information about the base component for each lane and the lane number is shown.

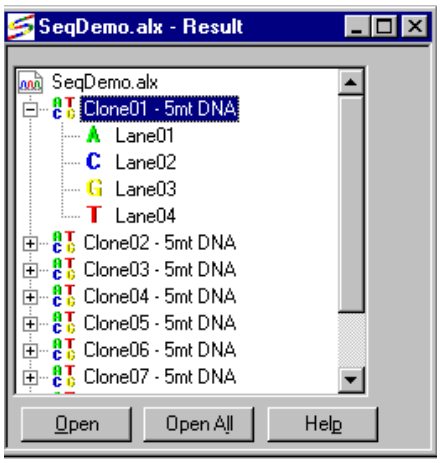


Figure 5-7. Result window showing the sequencing lanes for the first clone.

5.5 Presenting curves in the Curve window

The ability to view and manipulate the raw data curves and processed data curves in the **Curve** window is essential to the successful evaluation and editing of data using the various features of ALFwin Sequence Analyser. The **Curve** window gives a graphical representation of the curves for a selected clone. **Curve** windows can be opened for more than one clone at a time from within the same result file and from other opened result files. The Curve window contains an orientation view that shows the whole length of the run and allows you to quickly select any part of the run for closer viewing of the curves. If you have processed the data, by default the generated nucleotide sequence is displayed in this orientation view beneath the peaks of the curves.

5.5.1 Opening Curve windows

To open **Curve** windows:

1. Select the **Result** window and double click on a clone or select a clone and click on **Open**. To select a number of clones, hold down the <Ctrl> key while clicking on the clones required then click on **Open**. Alternatively, you can simultaneously open **Curve** windows for all of the clones by clicking on **Open All**. The **Curve** window(s) for the clone(s) are displayed one upon the other. If you want to view all the opened curves, select **Window:Tile All Curve Windows**.

   If you have more than one result file open in the ALFwin Sequence Analyser workspace, you can open any number of **Curve** windows from the **Result** windows.

2. Select the appropriate **Curve** window from the **Window** menu or arrange the display of all the curves (see Section 5.5.10).

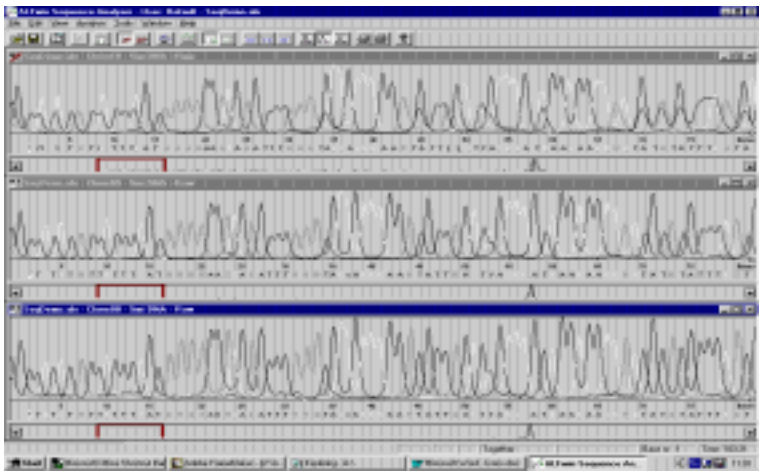


Figure 5-8. The Curve windows after selecting Till All Curve Windows. Use in conjunction with window linking to compare corresponding parts of the curves.

A **Curve** window contains two main views, the curve view (see Section 5.5.3) and the orientation view (see Section 5.5.4).

3. Select to view either the raw data curves or processed data curves.

5.5.2 Viewing raw or processed data curves

Curves can be viewed for clones that contain only raw data and also for clones that have been processed. You can tell whether a clone contains only raw data or has been processed by looking at the symbol in the top left corner of the **Curve** window (see Section 5.3.1). If the clone contains only raw data, some of the tools associated with the **Curve** window will not be available.

If the data have already been processed, it is possible to toggle between viewing the curves for the raw data and the processed data. To view the raw data curve, select either **View:Raw Data**, press the <F5> key or click on the **Raw Data** toolbar button. Conversely, to view the processed data curve, select either **View:Processed Data**, press the <F6> key or click on the **Processed Data** toolbar button.

See Section 5.7 for more information about processed data.

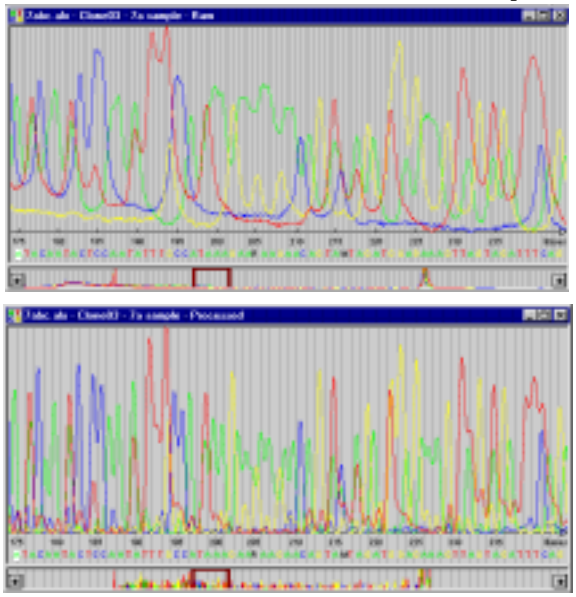


Figure 5-9. The two alternative views of the data curves, raw and processed. (To obtain these two simultaneous views for a clone, open the clone twice and then Tile All Curve Windows.)

5.5.3 Curve view

The curve view for a clone consists of four colour coded overlapping curves that represent the sequencing data for the four bases A, C, T, G. The curve represents the signals detected by the laser system in the ALF family instrument and each peak shows the presence of nucleotide

material in the sample.

If the data have been processed for the viewed clone, the calculated sequence is displayed directly beneath the curves. The order of the bases A, C, G, T in the sequence corresponds to the order of the peaks in the curves and are similarly colour-coded to the curves they represent. It is also possible to view ambiguity codes, which are the IUPAC codes denoting the presence of more than one base component at a single base position. Ambiguity codes are discussed in Section 5.7.1 and Appendix C. The bases of the sequence will consist of capital letters or small letters and can be either upright or italic.

Viewing curve legends

To view the legends showing which of the four bases the curves represent, select **View:Show Legends**. The legends are displayed on the right side of the curve view.

Mouse pointer position in Curves view

Click in the **Curves** view and the mouse pointer is accompanied by an information window which gives the position value for time. When the pointer touches a curve, the type of curve and its amplitude at the point of contact is displayed. If you zoom with the mouse (see Section 5.5.6) you can see the zoomed time interval in this display. This provides the opportunity to measure intervals/distances between peaks. If you point at a base letter, the information window displays the base number. To switch this information window off or on again, select **View:Show Info Window**.

Using the vertical cursor line

To display a vertical cursor line, select **View:Show Line**. The line moves left and right with the mouse pointer. The vertical cursor line allows more precise positioning of the cursor in relation to the curves and the time axis and can be used to position a base when editing the sequence.

5.5.4 Orientation view

The orientation view at the bottom of the window shows the whole result as curves, sequence bars or sequence text (3, 5 or 10 letter format). For further information, refer to Section 5.5.17. The rectangular cursor in the orientation view indicates the area of the result that is displayed in the curve view.

To alter the size of the orientation view:

1. Position the mouse pointer over its top border so that the pointer becomes a bi-directional arrow up and down.

2. Press down and hold the left mouse button and then drag the top border to increase or decrease the size of the orientation view.

3. Release the mouse button.

5.5.5 Navigating the Curve window

Use the orientation view to select different areas of the result to be displayed in the curve view.

1. Place the mouse pointer in the centre of the rectangular cursor, press and hold the left mouse button and drag the rectangular cursor back and forth to select the appropriate area of the curve.

2. Release the mouse button.

Alternatively, click in the orientation view outside of the rectangular cursor or use the page up/page down keys to scroll quickly. Use the left and right scroll arrows to scroll only a few bases at a time.

5.5.6 Using the zoom function in the Curve window

You can alter the magnification of the **Curves** view in the **Curves** window.

Changing magnification with the zoom commands

Command	*Effect*
View:Zoom In <F7> key	Successively increases the magnification.
View:Zoom Out <F8> key	Successively decreases the magnification.

Changing the magnification in orientation view

You can change the magnification of the curves by altering the size of the rectangular cursor in the orientation view.

1. Position the mouse pointer over the left or right edge of the rectangular cursor. The pointer becomes a bi-directional arrow to the left and right.

2. Press and hold the left mouse button and drag the border inwards or outwards. A larger rectangular cursor will reduce the magnification of the curves displayed in curve view and, conversely, a smaller rectangular cursor will increase the magnification of the curves.

3. Release the mouse button.

Resetting the magnification

To reset the magnification factor to the default level, select **View:Reset Zoom** or the <F9> key.

5.5.7 Stacking the curves

To stack the curves in the curve view, select (check) the **View:Stack Curves** command or click on the **Stack** button. Select either of these commands again to toggle back to overlapping curves.

5.5.8 Selecting individual base curves for viewing

By default, all the base components are shown in the curve view. Section 5.5.8 describes how to change this default display.

To view selected curves only in the curve view, deselect (uncheck) unwanted base components using **View:Include Curves**.

5.5.9 Viewing complementary or reverse curves

You can display the complementary and/or reverse curves for the run data.

Select (check) **View:Complement** to view the complementary data curve and/or select (check) **View:Reverse** to view the curve for reverse data. Alternatively, to simultaneously view complement and reverse data curves, **View:Comp and Rev** selects both. To return to the default view, select (uncheck) the option(s) again.

5.5.10 Arranging Curve windows

If you have opened several **Curve** windows, select the one that you want to view in the **Window** menu. Alternatively, there are several alternative ways to arrange the display of all the windows:

Command	*Effect*
Window:Cascade	This places all open windows on the ALFwin Sequence Analyser workspace on top of one another. The last selected window is displayed on the top of the cascaded windows.
Window:Tile All Windows	This displays all open windows on the ALFwin Sequence Analyser workspace, without overlapping.
Window:Tile All Curve Windows	This displays all open **Curve** windows on the ALFwin Sequence Analyser workspace side by side. This option is particularly useful for comparing **Curve** windows and can be used with the link function (see Section 5.5.11).

5.5.11 Linking Curve windows for comparison of clones

To aid in the comparison of curves, several **Curve** windows can be linked to compare regions of the result and simultaneously respond to a single command function.

1. Open the **Curve** windows that you want to compare and close all other **Curve** windows.

2. Select **Window:Tile All Curve Windows** to display all of the **Curve** windows on the screen.

3. Adjust the position and magnification of each curve to display the regions you wish to compare.

4. Select **Window:Link**.

5. Work with one **Curve** window, by zooming or moving within the curve and all the linked **Curve** windows will respond in the same way.

5.5.12 Changing the axes

X-axis units

1. Select any open **Curve** window.

2. Select **View:Properties** and click on the **Settings** tab in the **Curve Properties - Layout** dialogue.

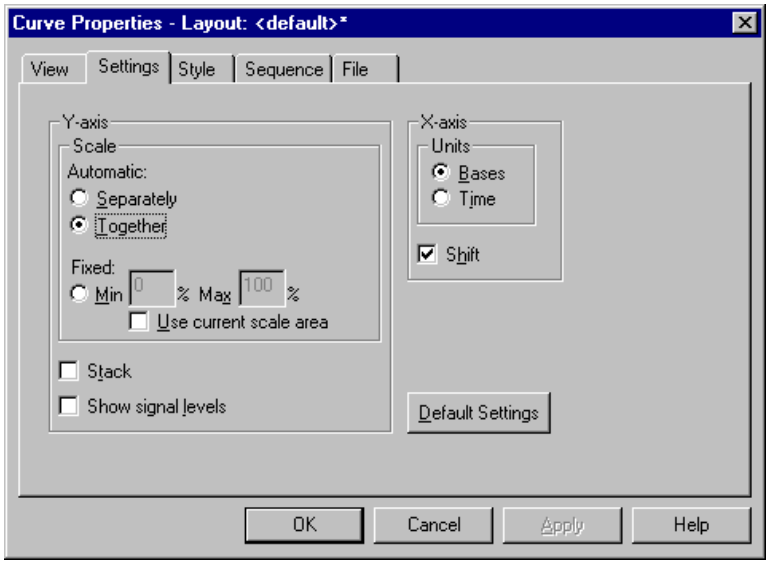


Figure 5-10. Settings tab of the Curve Properties - Layout dialogue.

3. Select the relevant **X-axis Units**, either **Bases** or **Time**.

4. Select **Shift** when you want to see the shift from the processing in all the curve windows or when you want to apply manual shift (see Section 5.7.5).

5. To effect the changes without closing the dialogue, click on **Apply**. To close the dialogue and effect the changes, click on **OK**.

   The changes are applied to all open **Curve** windows.

Y-axis scaling

To change the scale for the currently selected clone **Curve** window:

Command	Effect
View:Scale:Separately	This automatically scales each of the curves separately so that the displayed amplitude scale for each curve is adjusted to best fit the window displayed. This may be useful in discerning the peaks in a curve that had a relatively low detection signal during sequencing. Note that selection of separate scales can be misleading when, for example, trying to establish if a peak is a true peak or background noise.
View:Scale:Together	This automatically maximises the size of the largest peak visible in the current view of the **Curves** window. The amplitude scale for the curve containing this maximised peak, in the current view, is used as the basis to scale the remaining curves. Thus, all peaks are correctly proportioned in relation to the largest visible peak. This allows you to look at the relative signal detection sensitivity of the curves for the area of the result that you are viewing in the curve view.
View:Scale:Fixed	This displays the portion of the curve that corresponds to minimum and maximum amplitude limits that you have defined in the **Properties** dialogue (see below).

To change the scale for the open **Curve** windows:

1. Select any open **Curve** window.
2. Select **View:Properties** and click on the **Settings** tab in the **Curve Properties-Layout** dialogue.

3. Select the relevant **Y-axis Scale**, either **Separately** or **Together** (see above), or **Fixed**. If you select **Fixed**, you must enter a minimum and maximum amplitude value, e.g. an entered range of 0% to 50% means that the portion of the curves with amplitude between 0% and 50% of the possible range is shown.

4. If you want the scale to be fixed according to the default scale area of the automatically calculated scales, i.e. as calculated for **Separately** or **Together**, check the **Use current scale area** box. Whenever you select **Fixed**, you can scroll along the result and view the peaks at their real height relative to the maximum amplitude for the whole run.

5. To effect the changes without closing the dialogue, click on **Apply**. To close the dialogue and effect the changes, click on **OK**.

5.5.13 Changing the presentation of the curves within the curve windows

1. Select any open **Curve** window.

2. Select **View:Properties** and click on the **Settings** tab in the **Curve Properties-Layout** dialogue.

3. Select (check) **Stack** to stack the curves. Leave this option unchecked to display overlapping curves.

4. To effect the change without closing the dialogue, click on **Apply**. To close the dialogue and effect the change, click on **OK**.

5.5.14 Showing the signal levels for the currently displayed curve

1. Select any open **Curve** window.

2. Select **View:Properties** and click on the **Settings** tab in the **Curve Properties-Layout** dialogue.

3. To display the **Signal Level** table, that shows the minimum and maximum amplitudes and the differences in amplitude for the part of the curves currently displayed, select (check) the **Show signal levels** option.

4. To effect the change without closing the dialogue, click on **Apply**. To close the dialogue and effect the change, click on **OK**.

5.5.15 Changing the default setting for the base curves shown in curve view

1. Select any open **Curve** window.

2. Select **View:Properties** and click on the **View** tab in the **Curve Properties-Layout** dialogue.

3. By default, all four base curves are shown in a curve view. To change this default setting, select the base curves you require by highlighting them in the **Base curves** field.

4. To effect the change without closing the dialogue, click on **Apply**. To close the dialogue and effect the change, click on **OK**.

5.5.16 Changing the default setting for the type of data curve displayed in curve view

1. Select any open **Curve** window.

2. Select **View:Properties** and click on the **View** tab in the **Curve Properties-Layout** dialogue.

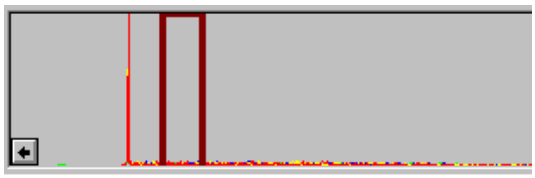
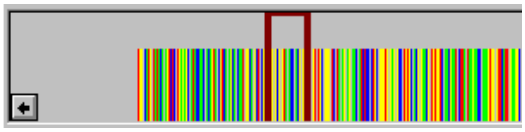
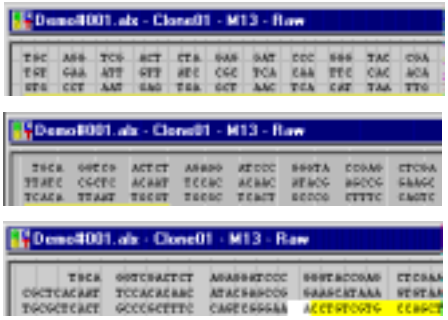
3. Select the default **Dataset** required, **Raw** or **Processed**.

4. To effect the change without closing the dialogue, click on **Apply**. To close the dialogue and effect the change, click on **OK**.

5.5.17 Changing modes in orientation view

If the raw data for a selected clone have been processed, it is possible to view sequence information in three different modes within the orientation view of the **Curves** window.

To change mode for one clone, select the **Curves** window for that mode and click anywhere in the orientation view. Select one of the following:

Command	*Effect*
View:Curves This is the default mode.	 *Figure 5-11.* Orientation view, Curves.
View:Sequence Bar In this view, ambiguities are shown with higher bars than other bases. Use this to help find ambiguity positions.	 *Figure 5-12.* Orientation view, Sequence Bar.
View:Sequence Text: **Group 3** **Group 5** **Group 10** Sequence text can be viewed in groups of three, five or ten bases depending on the selected option.	 *Figure 5-13.* Orientation view, Sequence Text, grouped 3, 5, 10 from top to bottom.

To change mode for all open **Curve** windows do the following:

1. Select any open **Curve** window.

2. Select **View:Properties** and click on the **View** tab in the **Curve Properties-Layout** dialogue.

3. Select the appropriate orientation view.

4. To effect the changes without closing the dialogue, click on **Apply**. To close the dialogue and effect the changes, click on **OK**.

5.5.18 Including instrument data as curves

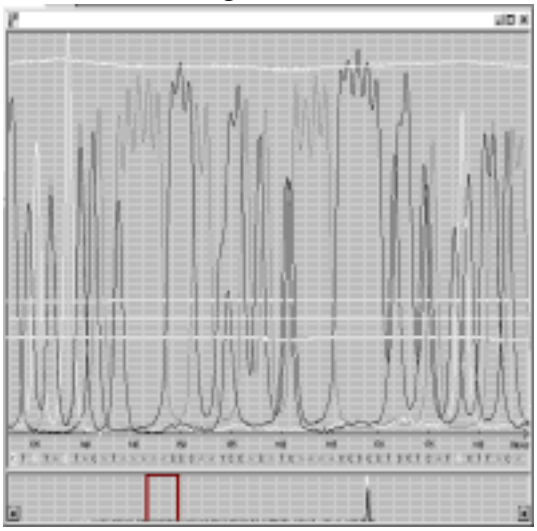


Figure 5-14. Instrument data curves shown below stacked data curves.

To include instrument data as curves:

1. Click anywhere in the appropriate **Curve** window.

2. Select **View:Properties** and click on the **View** tab in the **Curve Properties-Layout** dialogue.

3. Select the required **Instrument data**. You can select an individual item, specific items if you hold down the <Ctrl> key while you select them, or a range of items if you press the <Shift> key while selecting the last item.

4. To effect the changes without closing the dialogue, click on **Apply**. To close the dialogue and effect the changes, click on **OK**.

To check values of the instrument data:

1. Select the relevant **Curve** window.

2. Place the mouse pointer on the **Instrument data** curve of interest. The value at the pointer is shown in the Status Bar. Note that the scale of the instrument data is fixed.

5.5.19 Changing the font and colours in the Curve window

You can change the colour of the curves and the background. You can also change the font and the curve width.

To change colours:

1. Select the relevant **Curve** window.

2. Select **View:Properties** and click on the **Style** tab in the **Curve Properties-Layout** dialogue.

3. To change colour, select the appropriate item under the **Colour** list and click on the **Set Colour** button. The **Colour** dialogue is shown.

4. Select a colour.

5. Click on **OK**.

Note: Only solid colour will be printed or copied to the clipboard.

6. Select a curve width. Double click in the **Curve width** field and enter the line thickness required in the range of 1 to 50 pixels.

7 To effect the changes without closing the dialogue, click on **Apply**. To close the dialogue and effect the changes, click on **OK**. The colour is changed in all **Curve** windows.

To change the font:

1. In the **Style** page of the **Curve Properties-Layout** dialogue, select the **Set Font** button. The **Font** dialogue is shown.

2. Select a **Font**, **Font style** and **Size**.

3. Click on **OK**.

4. To effect the changes without closing the dialogue, click on **Apply**. To close the dialogue and effect the changes, click on **OK**.

If you want to restore the default settings, click on the **Default Settings** button.

5.5.20 Additional information in the curve window

You can configure the Curve window to show:

legends	that labels each curve with their base type
a vertical cursor line	that assists positioning within a **Curve** window
an information window	that displays curve information alongside the mouse pointer in the curve view. Details are provided in Section 5.5.3.

To configure these options on or off:

1. Click anywhere in the appropriate **Curve** window.

2. Select **View:Properties** and click on the **Style** tab in the **Curve Properties-Layout** dialogue.

3. Select the items required from the **Style** options. Deselect those items not required.

4. To effect the changes without closing the dialogue, click on **Apply**. To close the dialogue and effect the changes, click on **OK**.

5.5.21 Configuring the sequence display

You can configure the way in which base sequences are shown and edited in the **Curve** window.

1. Click anywhere in the appropriate **Curve** window.

2. Select **View:Properties** and click on the **Sequence** tab in the **Curve Properties-Layout** dialogue.

3. Select/deselect the options available:

Edit Sequence	to allow/disallow editing of sequence bases
Show Edited Bases	to show/hide underlining of edited bases
Show Italics	to show italic bases or display them as upright bases
Complement	to show the complement/normal sequence
Reverse	to show the reverse/forward sequence

5.5.22 Saving or opening a layout for the Curve window

You can save a **Curve** window layout for later use. All user-definable properties of the curve window are saved.

To save a layout or open a saved layout:

1. Select the relevant **Curve** window.

2. Select **View:Properties** and click on the **File** tab in the **Curve Properties-Layout** dialogue.

3. Use the following functions from the **File** tab:

Save Layout As button	Allows you to save the layout for later use as a **.lyt** file. The last used layout file will be applied by default the next time ALFwin Sequence Analyser is started by the same user.
Open Layout button	Allows you to open a saved layout.

4. Click on **OK**.

5.6 Presenting the run in the Gel View window

To open the **Gel View** window, select **View:Gel** or click on the **Show Gel** button.

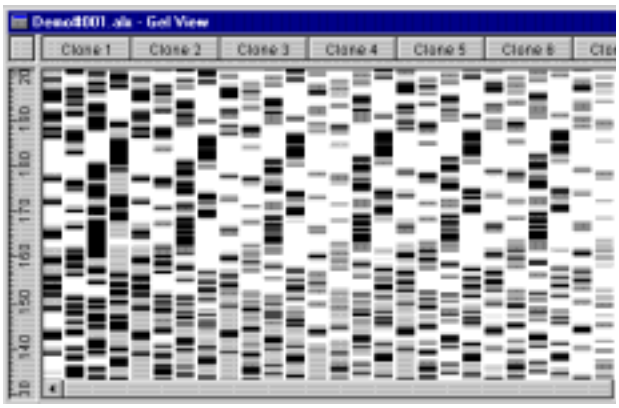


Figure 5-15. Gel View window.

The **Gel View** window gives a pictorial representation of the bands in each lane in the sequencing gel. These can be used to check the primer peaks, background noise and signal levels. Primer peaks are shown as black bands in the lower part of the gel, whereas the sample bands are shown higher up in the gel in different grey scale densities dependent on the strength of the signals.

5.6.1 Viewing raw data or processed data

If the data have already been processed, it is possible to toggle between viewing the processed data and the raw data. To view the raw data, select either **View:Raw Data**, press the <F5> key or click on the **Raw Data** toolbar button. Conversely, to view the processed data, select either **View:Processed Data**, press the <F6> key or click on the **Processed Data** toolbar button.

By default, the gel view first shows the raw data. You can change this default:

1. Select the **Gel View** window.

2. Select **View:Properties** and click on the **Data Set** tab in the **Gel Properties** dialogue.

3. Select the dataset required, **Raw data** or **Processed data**.

4. To effect the change without closing the dialogue, click on **Apply**. To close the dialogue and effect the change, click on **OK**.

5.6.2 Navigating the Gel View window

Use the scroll bars to display different portions of the gel.The position of the mouse pointer is indicated in the status bar.
The vertical position is shown as time into the run
The horizontal position is shown in the form Clone number:base type. eg Clone7:A.

5.6.3 Using the Zoom function in Gel View

You can alter the magnification of the whole gel view or select particular portions of the gel using the mouse for increased magnification.

Altering the magnification of the whole gel

To alter the magnification of the whole gel, use the following commands:

Command	*Effect*
View:Zoom Time:In x2 <F7> key	Increases the magnification of the time scale by a factor of 2.
View:Zoom Channels:In x2 <Shift + F7> keys	Increases the magnification of the gel lanes by a factor of 2.
View:Zoom Time:Out x2 <F8> key	Decreases the magnification of the time scale by a factor of 2.
View:Zoom Channels:Out x2 <Shift + F8> keys	Decreases the magnification of the gel lanes by a factor of 2.

Increasing the magnification using the mouse

To magnify a specific portion of the **Gel View** window using the mouse:

1. Select either **View:Zoom with Mouse**, the <F10> key or click on the **Zoom with Mouse** toolbar button.

2. Position the mouse pointer in the top left corner of the area you want to magnify in the **Gel View** window. Press and hold the left mouse button and drag out a box to encompass the area that you want to magnify. Release the left mouse button.

Resetting the magnification

To reset the magnification factor to the default level, select either **View:Reset Zoom**, the <F9> key or click on the **Reset Zoom** toolbar button.

5.6.4 Adjusting the Gel View window properties

Adjusting the Gel View contrast

You can adjust the contrast of the **Gel View** window to improve visualisation of the bands in the lanes.

1. Select **View:Gel Properties**. The **Gel Properties** dialogue is displayed. Click on the **Data Set** tab.

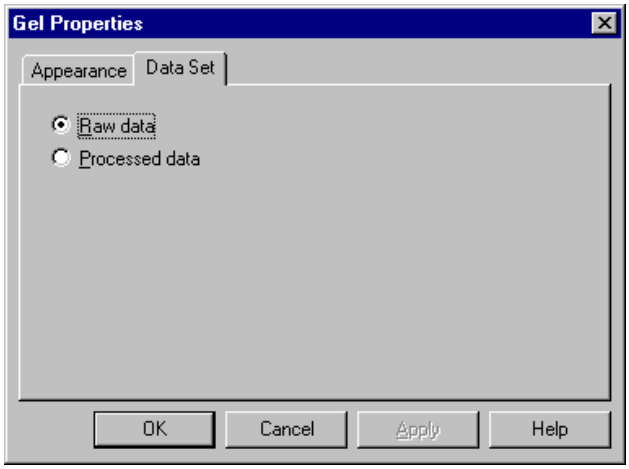


Figure 5-16. Data Set tab of the Gel Properties dialogue.

2. If necessary, you can select the data set in which the contrast changes are to be made. Select the appropriate data set, either **Raw data** or **Processed data**. The data displayed in the **Gel View** window are correspondingly displayed.

3. Select the **Appearance** tab.

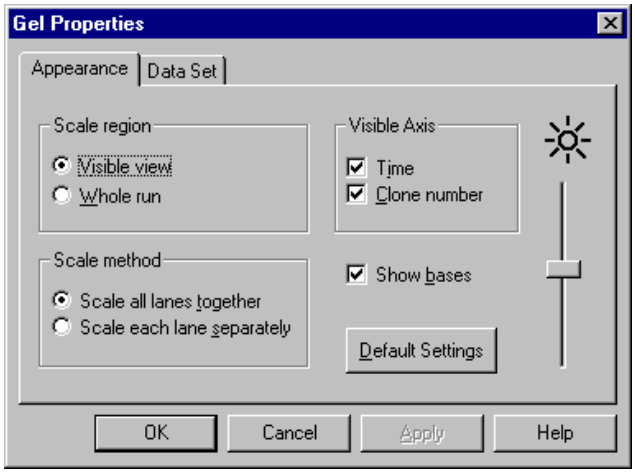


Figure 5-17. Appearance tab of the Gel Properties dialogue.

4. Select the **Scale region** to apply the contrast adjustment, either to the **Visible view** on the screen or to the **Whole run**.

5. Select the **Scale method**, the mode of calculation for the contrast adjustment, either:

Scale all lanes together	This applies an adjustment in contrast to the lanes in the selected region (see step 4) based on contrast data from all of the lanes.
Scale each lane separately	This applies an adjustment in contrast to individual lanes based on their own contrast data.

6. Adjust the contrast by dragging the slide bar up or down for increased or decreased contrast respectively.

7. Click on **Apply** to effect the change. Readjust the contrast level if required or click on **Default Setting** to return all the settings to their default conditions.

8. When you are satisfied with the adjusted contrast level, click on **OK**.

Showing and hiding the Gel View axes and bases

You can adjust the **Gel View** to show or hide a vertical time axis and a horizontal clone number axis. You can also show or hide the base positions evaluated by data processing.

1. Select **View:Gel Properties**. The **Gel Properties** dialogue is displayed.

2. Click on the **Appearance** tab.

3. Select or deselect **Time** and **Clone number** according to the axes you want to display on the borders of the **Gel View**.

4. Select or deselect **Show bases**. When this option is selected, the base positions are superimposed, in a different colour, on the Gel lanes of processed data.

5. Click on **Apply** to effect the change without closing the dialogue.

6. When you are satisfied with the settings, click on **OK**.

5.7 Processing run data

The key purpose of the ALFwin software application is that a nucleotide sequence can be extracted from the raw sequencing data. The algorithms used to calculate the nucleotide sequence (see Appendix C) are extremely accurate in calling the correct order of bases as they were detected on the gel, but also sensitive in being able to sort a real peak out from base signal levels and to differentiate individual peaks from close running fragments in the gel.

ALFwin Sequence Analyser allows you to process the raw data with various parameters applied to improve the quality of the result. Such parameters include being able to set the stringency level of the data processing based on the degree of heterozygocity expected in the samples, or to manually set the start and stop time for the data included in the processing. Following data processing, various numeric values are given that give information and clues about the success of the run. Viewing the data curve directly from the processing dialogues requires only a double click of the mouse button. Working in the **Curves** window for a clone, you can easily view the data and directly edit base nucleotides on the screen.

When you work with data processing, you will be working with five types of curves:

- raw data before processing - no bases called
- raw data after processing - bases called
- shifted raw data
- processed data
- shifted processed data.

5.7.1 Processing clones

To process raw data:

1. With a result file open, select **Analysis:Process**. The **Process Status** dialogue opens:

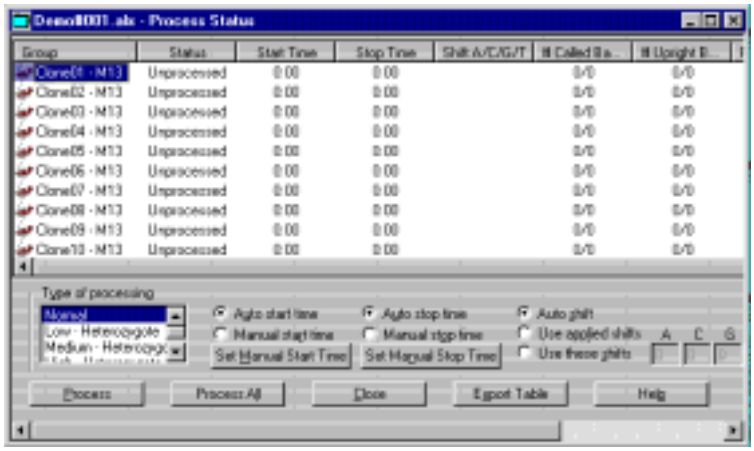


Figure 5-18. Process Status dialogue.

2. Select the clone(s) to be processed. This is not necessary if you are going to process all the clones at the same time.

To select more than one clone in a sequence	Click on the first clone, hold down the <Shift> key and click on the last clone in the column.

To select a number of separate clones	Hold down <Ctrl> and click on the clones required.

Note: It is useful to select all the clones of interest in the **Result** window. Then these will be preselected in the **Process** window.

3. Select the type of processing to be applied using the options available in the **Type of processing** field. Only one type of processing is allowed at a time. Select **Normal** when no heterozygous positions are expected. Otherwise, select one of the heterozygote modes depending on how sensitive the algorithm should be to ambiguity codes:

Low-Heterozygote	There is a low expectancy of finding ambiguity codes
Medium-Heterozygote	There is a medium expectancy of finding ambiguity codes
High-Heterozygote	There is a high expectancy of finding ambiguity codes.

4. Select **Auto Start Time** so that processing starts after the highest found (primer) peak or after a stop or where the raw data curves have a normal form.

5. Select **Auto Stop Time** so that processing stops after the last found peak.

   If the program is unable to make a normal evaluation using the auto settings, you can try using manual start time. You can also use manual stop time. The procedure is described in Section 5.7.6.

6. Select **Auto Shift** so that the software calculates the shifts to be used. If the shift of lanes in the gel is too large to be corrected automatically by the program, it will be necessary to shift the curves manually. This procedure is described in Section 5.7.5.

7. Click on **Process** to execute base calling on the selected clones or click on **Process All** to process all the clones. To halt processing at any point, press the <Esc> key.

8. The progress of the raw data processing can be observed in the **Status** column of the **Process Status** dialogue. When the status of

all the selected clones has changed, raw data processing is complete.

5.7.2 Reviewing the data processing results

You can view the results of the data processing and obtain confirmation that the run proceeded successfully.This review provides confirmation about the whole experiment including:

- the sample
- sample preparation
- the run
- data processing to produce the nucleotide sequence.

A particularly useful indicator is the ratio of upright bases to the total of called bases, and also the total number of called bases between adjacent clones of the same sequenced region.

1. In the **Process Status** dialogue, look at the column of data labelled **#Called base/Ambiguities** and the column labelled **#Upright bases/ Ambiguities**. Compare the first values in each of the columns for a clone and compare with the values in an adjacent clone. Compare how many of the total number of called bases are upright bases between the two clones:

# Called Bases/Ambiguities	# Upright Bases/Ambiguities
1157/58	856/1
1037/56	737/10
1100/71	798/10
1096/60	794/2
1066/51	764/3
911/81	610/6
972/86	672/7
884/85	584/8
573/64	273/6
1021/89	720/10

Figure 5-19. Comparing called and upright bases in adjacent clones with different samples.

Called bases are the bases identified in the raw data by the base calling algorithm during processing. Particularly at the very end of a sequencing run, when the electrophoretic separation performs less well, the bases are more difficult to detect by the base calling algorithm so are assigned as italic bases.

2. Compare the total number of called bases between adjacent clones from the same sample.
   If there is an obvious disparity between the values then you should check the data curves and the nucleotide sequence.

5.7.3 Viewing the data curve after processing and finding special sequences

You can view the data curve for a processed clone directly from the **Process Status** dialogue. It is important that you know how to work in the **Curves** window to make the best of your viewing and evaluation (see Section 5.5). You can use the **Curves** window to find special sequences or ambiguities.

Viewing the data curves after processing/base calling

1. Double click on the clone icon in the **Process Status** dialogue. The **Curves** window containing the data curves for the selected clone is displayed beneath the dialogue.

2. Use View:Raw Data or click on the raw data button. The base nucleotide sequence is displayed underneath.

3. Use **View:Processed Data** or click on the **Processed Data** button to display curves for the processed data. The base nucleotide sequence is displayed underneath.

   The sequence comprises the colour coded base nucleotide letters A,C,G and T, in addition to any detected base ambiguity codes, i.e. a base position where more than one base component was called by the algorithm. See Appendix C for information of the ambiguity codes.

   The sequence also comprises different styles for representing the base nucleotides. Bases are written in different styles depending on how the algorithm has evaluated the base position. Please refer to Appendix C which describes the operation of the base calling algorithm:

CAPITAL letter	The algorithm estimates that it is likely that the base has an uncertainty of less than one percent
Italic letter	The algorithm estimates that it is likely that the base has an uncertainty greater than one percent
small (lower case) letter	The algorithm is uncertain whether or not there is a base at that position.

4. Use the rectangular cursor in the orientation view beneath the curves to select an area of the run and adjust its magnification. (Section 5.5.5 describes how the rectangular cursor is used to adjust the curve view.)

5. Use the <F7> and <F8> keyboard keys to adjust the magnification of the view or use the mouse pointer to drag a box around the area of the curves to be magnified.

Finding special sequences or ambiguities

To find the positions of special sequences or ambiguities:

1. Click on the **Curve** window to make sure that it is activated.

2. Select **Edit:Find** or press the <Ctrl+F> keys. The **Find** dialogue is shown.

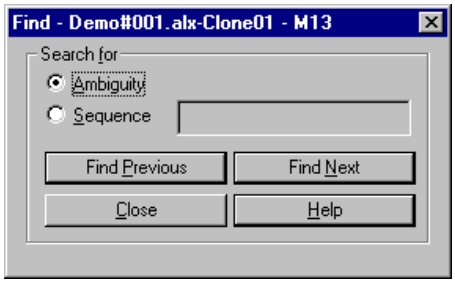


Figure 5-20. Find dialogue.

3. Select **Ambiguity** or **Sequence**.
If **Sequence** is selected, type in the **Sequence** field the sequence required. For both forward and reverse curves, start with the base

with the lowest number in the sequence.

4. Click **Find Previous** to search to the left in the curves or click **Find Next** to search to the right. The first found ambiguity/sequence is highlighted in the **Curve** window.

5. Click **Find Previous** or **Find Next** again to continue the search.

6. Click **Close** to end the search.

5.7.4 Assigning upright status to bases

If you want to assign upright status to bases that the processing algorithm has calculated with less certainty, namely italic bases or small bases:

1. Display the raw or processed data curves and sequences for the appropriate clone either from the **Process Status** dialogue or the **Result** window.

2. Select **Edit:Mark Upright Bases**. The **Mark Upright Bases** dialogue is shown.

3. Click in the **Start** field and enter the number of the first base to be marked by typing or by clicking on the start point in the **Curve** window.

4. Click in the **End** field and enter the number of the last base to be marked or click on the end point in the **Curve** window.

5. Click **OK** to mark all the selected bases as upright bases.

   Note: As an alternative to steps 3 - 4 in the above procedure, bases can be marked directly in the **Curve** window.

Hiding the italic status of bases

You can choose to view italic bases as upright bases by hiding the italic status.

1. Click on the **Curve** window to make sure that it is activated.

2. Deactivate italic status by selecting **View:Show Italics**. This removes the check mark from this menu option meaning that it is not active.

All bases are shown as upright bases. Repeat the above procedure to activate the menu command to show italic bases.

Note: The italic status is only hidden from view despite all bases being displayed as upright bases. For processing purposes, italic bases are always so unless they are *assigned* upright status.

5.7.5 Shifting the curves manually

When there are few upright bases in a processed clone, the cause may be excessive shift of one or more lanes in the gel. For example, the effect known as "smiling" is produced when samples in the first and last lanes move more slowly than those in the middle lanes. This may be evident when viewing the primer peaks for the four curves of a clone. You can apply manual shift correction to make it easier for the program to analyse the run.

To shift a curve by applying manual shifts:

1. Click on the **Curve** window to make sure that it is activated.

2. Zoom in to the curve so that adjacent peaks can be seen clearly.

3. Select either **Edit:Apply Shift** or <Ctrl+Y> keys. The **Shift Toolbar**, with four arrow buttons and four lane buttons, is added to the other buttons in the toolbar:

Figure 5-21. Shift toolbar.

You can move the Shift Toolbar to another position on screen if any toolbar buttons are obscured. Click and hold on a non-button part of the toolbar and drag it to the position required.

4. Select the appropriate lane (**A**, **C**, **G** or **T**) button.

5. Move the selected lane curve by clicking the left or right arrow button, adjusting until the distances between adjacent peaks are even.

6. From the **Process Status** dialogue, select **Use applied shifts**.

7. Click on **Process** to process the selected clone.

Recalling previously applied shifts

Manual shifts that have been applied to a curve are recorded in the Analysis Logbook. You can recall these records using **Edit:Casebook** and selecting the **Analysis Log** tab.

To reproduce the same shifts:

1. From the **Process Status** window, select **Use these shifts**.

2. Enter the values for the shifts in the **A**, **C**, **G** and **T** fields.

3. Click on **Process** to process the selected clone.

Please refer to Appendix C for further information about shift.

5.7.6 Using manual start or stop times

If a normal sequence evaluation can not be obtained using auto selection of start and stop times, it may help if you manually set these times before processing the raw data. You may use a combination of auto and manually set start and stop times.

1. Select the clone or clones and select **Manual start time**.

2. Click on the **Curve** window and select **View:Show Line** to switch on the vertical line that allows more precise positioning of the cursor in relation to the curves and the time axis.

3. Click the **Set Manual Start Time** button and click in the **Curve** window at the position where processing is to start. The time reading is transferred to the **Start Time** column for the selected clones. To change the selected time, click again in the **Curve** window. You can use the time display that accompanies the cursor to help position it correctly.

4. Repeat the above procedure to set the **Manual stop time**.

5. Click on **Process** to process the selected clones or click on **Process All** to process all the clones.

5.7.7 Displaying the signal levels of lane curves

Signal levels are measured as a percentage of the maximum possible amplitude.

To display the amplitudes of the lane curves:

1. Click on the **Curve** window to make sure that it is activated.

2. Adjust the **Curve** window to display the part of the curve required.

3. To display the **Signal Level** table for only the currently selected Curve window, select **View:Signal Levels** or press the <Ctrl+Shift+S> keys.
   To display the **Signal Level** table for all **Curve** windows, select **View:Properties** to open the **Curve Properties - Layout** dialogue. Select the **Settings** tab and check the **Show signal levels** option.

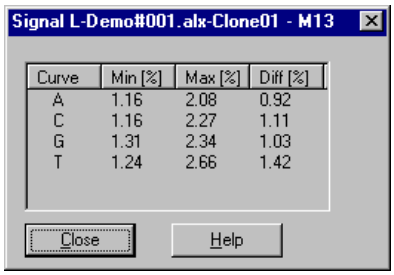


Figure 5-22. Signal Level table.

The table shows the minimum and maximum amplitudes and the differences in amplitude for the part of the curves currently displayed. This should allow you to gauge the minimum and difference in amplitude in one curve against the maximum peak amplitude in another curve.

5.8 Editing sequence bases

This section describes how you can edit the processed data to replace, insert, move or delete bases. All the changes you make are logged in the Analysis Log in the casebook.

You can edit bases using a combination of mouse and keyboard or you can use the mouse with the **Edit Toolbar**.

Warning:
Care is needed when selecting an edit mode. An active mode is indicated in the **Edit** menu by a black dot marker. Selecting a mode toggles it on or off. If you select a mode that is already active you can inadvertently turn it off and return to the default **Replace** mode. Check by looking in the status bar which shows the current edit mode.

The **Edit Toolbar**, is displayed on screen when you select **Edit:Edit Sequence** or press the <Ctrl + E> keys.
You can move the Edit Toolbar to another position on screen if any toolbar buttons are obscured. Click and hold on a non-button part of the toolbar and drag it to the position required.
This toolbar allows you to replace, insert, move and delete bases without calling these functions separately. Clicking the appropriate button on the **Edit Toolbar** executes action on the selected base:

Use a button or combination of buttons to set the type of the selected base.

Insert a base to the left of the currently selected base and select the new base.

Delete the currently selected base and select the next base to the right.

Move the currently selected base to the left.

Move the currently selected base to the right.

Undo the last action.

Edited clones are identified by a pen symbol in their ACGT icons in the **Result** window. Edited bases are underlined in the **Curve** window if **Show Edited Bases** is selected in the **View** menu or in the **Sequence** tab of the **Curve Properties - Layout** dialogue.

5.8.1 Replacing a base

To replace a base:

1 Click on the **Curve window** to make sure that it is activated.

2. Select **Edit:Edit Sequence** or press the <Ctrl+E> keys. **Replace Base** is selected as default and the **Edit Toolbar** is shown.

3. Move the mouse pointer over the sequence base in the **Curve** window. The pointer turns into a text caret. Click on the base component letter to select it.

4 Replace the base component by clicking on the appropriate base component button in the toolbar or type the base letter. Selecting more than one base component will automatically give the appropriate ambiguity code (see Appendix C). The base component buttons toggle between on and off for the respective bases (at least one base must be on).

5. You can undo the most recent replace by selecting **Edit:Undo Replace Mode** or by pressing the <Ctrl+Z> keys or by clicking the **Undo** button on the **Edit Toolbar**.

6. To exit edit mode, select **Edit:Edit Sequence to deactivate it** or click on the **Close** button in the **Edit Toolbar**.

5.8.2 Inserting a base

To insert a base in the sequence:

1. Click on the **Curve** window to make sure that it is activated.

2. Select **Edit:Edit Sequence** or press the <Ctrl+E> keys to activate the edit mode. The **Edit Toolbar** is shown.

3. Select the base to the right of the position where you want to insert a base.

4. Click the **Insert New Base** button on the **Edit Toolbar** and use the **ACGT** buttons to set the new base type. If you prefer to use the keyboard, select **Edit:Insert Base Mode** if it is not already activated then type the base letter.

5. You can undo the most recent base component insertion by clicking the **Undo** button on the **Edit Toolbar** or by selecting **Edit:Undo Insert**, or press the <Ctrl+Z> keys.

6. To exit edit mode, select **Edit:Edit Sequence** to deactivate it or click on the **Close** button in the **Edit toolbar**.

To insert a base using the mouse:

1. Select **Edit:Insert Base Mode**.

2. Select **Edit:Show Line** if the vertical cursor line is not already activated. This will help you locate the base correctly.

3. Select the base required on the **Edit Toolbar** then double click on the position on the curve where you wish to make the base insertion.

To insert bases to the end of a sequence:

You can use the mouse method described above to add bases to the end of a sequence. Alternatively:

1. Select the last base in a sequence.

2. Insert the same base.

3. Select the last base using the cursor key and move it to the right by using <Ctrl + right cursor key>.

4. Insert the first additional base.

5. Select the last base again and shift it to the right as in 3. above.

6. Insert the next additional base.

7. Repeat steps 5. and 6. until all the additional bases have been inserted.

8. Delete the last base.

5.8.3 Moving a base

To adjust the position of a base to fit the curve better:

1. Click on the **Curve** window to make sure that it is activated.

2. Select **Edit:Edit Sequence** or press the <Ctrl+E> keys to activate the edit mode. The **Edit Toolbar** is shown.

3. Select the base to be moved.

4. Move the selected base by dragging it with the mouse or by clicking the **move base right/left** buttons on the **Edit Toolbar** or by using the <Ctrl+cursor> keys (right/left).

5. You can move the last moved base back to its original position by clicking the **Undo** button on the **Edit Toolbar** or by selecting **Edit:Undo Move** or press the <Ctrl+Z> keys.

6. To exit edit mode, (deactivate) **Edit:Edit Sequence** or click on the **Close** button in the **Edit toolbar**.

5.8.4 Deleting a base

To delete a base:

1. Click on the **Curve** window to make sure that it is activated.

2. Select **Edit:Edit Sequence** or press the <Ctrl+E> keys to activate the edit mode. The **Edit Toolbar** is shown.

3. Select the base to be deleted.

4. Delete the base by clicking on the **Delete Base** button on the **Edit Toolbar** or by selecting **Edit:Delete Base** or by pressing the <Del> key.

5. You can undo the most recent delete by clicking the **Undo** button on the **Edit Toolbar** or by selecting **Edit:Undo Delete** or by pressing

the <Ctrl+Z> keys.

6. To exit edit mode, select **Edit:Edit Sequence** to deactivate it or click on the **Close** button in the Edit toolbar.

5.8.5 Showing or hiding edited bases

Edited bases are underlined by default. (Section 5.8.5 describes how you can change this default setting.) To hide this underlining:

1. Click on the **Curve** window to make sure that it is activated.

2. Select **View:Show Edited Bases**. The underlining is hidden. Repeat the above to show the underlining again.

5.8.6 Cutting clone curves

The first and last parts of a run do not always give meaningful information. To reduce the size of the result file, you can cut selected parts of the clone.

Warning:
When you cut a clone and save the file, the deleted data is lost. Take care when making a cut. It is prudent to save the file before applying a cut so that you can, if necessary, later return to the saved uncut version.

To cut a clone:

1. Select the clone to be cut and click on its **Curve** window to make sure that it is activated.

2. Select **Edit:Cut Clone**. The **Cut Clone** dialogue is shown.

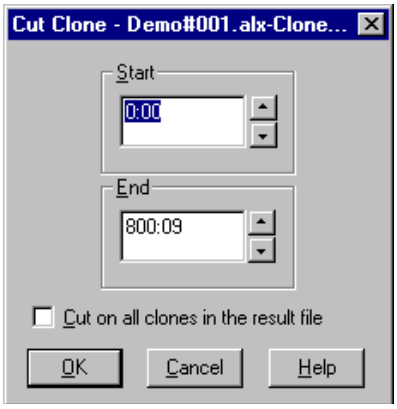


Figure 5-23. Cut Clone dialogue.

3. Use this dialogue to set the time range of the part of the clone that will be preserved. Data outside the limits set in the **Start** and **End** fields will be deleted.
   Click in the **Start** field and enter the start time in minutes and seconds. You can enter the time by typing or you can click on the start point in the **Curve** window. Positioning in the **Curve** window can be aided by first selecting **View:Show Line** to display a vertical cursor line at the mouse pointer.

4. Click in the **End** field and enter the time as above.

5. Select **Cut on all clones in the result file** if you want to apply the same cut to all the clones in the result file.

6. Click on **OK**. The **Verify Cut Clone** warning is shown.

7. Click on **OK** to cut the clone. If you wish to abandon a cut before confirming **OK**, click on **Cancel** in the **Cut Clone** or **Verify Cut Clone** dialogues. If you wish to reverse a cut that you have made, close the file without saving it and then open it again. Any unsaved changes you have made to the file will be lost.

5.9 Viewing casebook information and adding evaluation notes

A copy of the casebook information is included in the result file saved at the end of a run. This includes the record of all events which is stored in the run and evaluation logs. In the casebook copy, you can:

check which **Post-run Actions** were selected

read the **Run conditions**

read, add or change **Sample Information**

read the result **File Information**

read the **Pre-run** and **Run notes**

write **Analysis notes**

read the **Logbook** and **Analysis Log.**

To view or edit the copy of the Casebook in the result file:

1. Select **Edit:Casebook** or click the **Edit Casebook** button. The **Casebook** dialogue is shown:

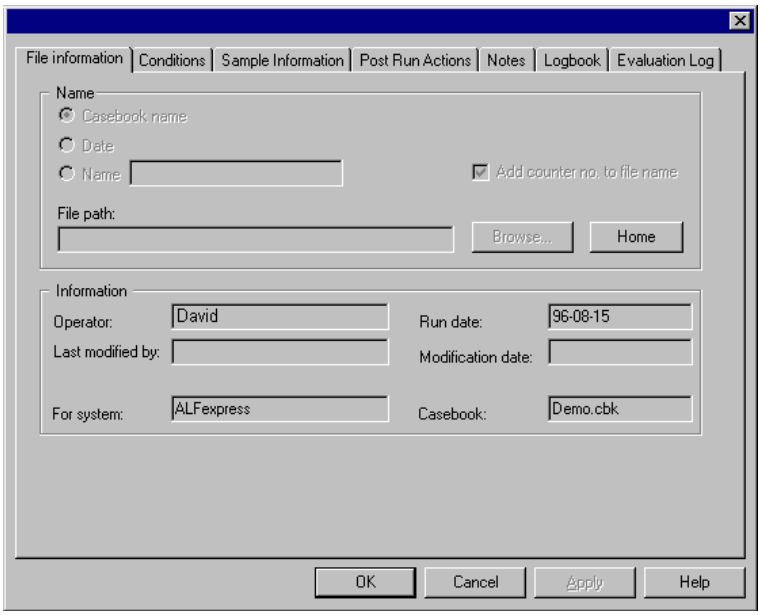


Figure 5-24. Casebook dialogue.

2. Select the **Sample Information** or **Notes** tabs to change or add information in the casebook.

3. Use the remaining tabs to access read-only information in the Casebook.

4. Click on **OK** and the changes and additions will be saved when you save the result file.

5.10 Saving the result file

ALFwin Sequence Analyser allows you to save the result file as one complete file or in two or more parts. Normally you save as one file but you may want to split the file so that only the part(s) of interest are saved. File splitting is also useful when you want to accommodate a large result file on media of limited capacity such as diskettes.

5.10.1 Saving a complete result file

To save the result file in the same location and using the existing file name, select **File:Save**.

To save using a different name or location:

1. Select **File:Save As**. The **Save As** dialogue opens.
2. Use the dialogue to select the folder where the file is to be saved.
3. Enter a name for the file.
4. Click on **Save**.

5.10.2 Saving part(s) of a result file

A result file can be split into its clone data components. You select which clone(s) are saved and whether they are saved in one file or in a separate file for each clone. Files are normally saved in .alx format but you can choose to save in .alf format if required.

To save part(s) of a result file:

1. Select **File:Split File**. The **Split File Settings** dialogue opens.

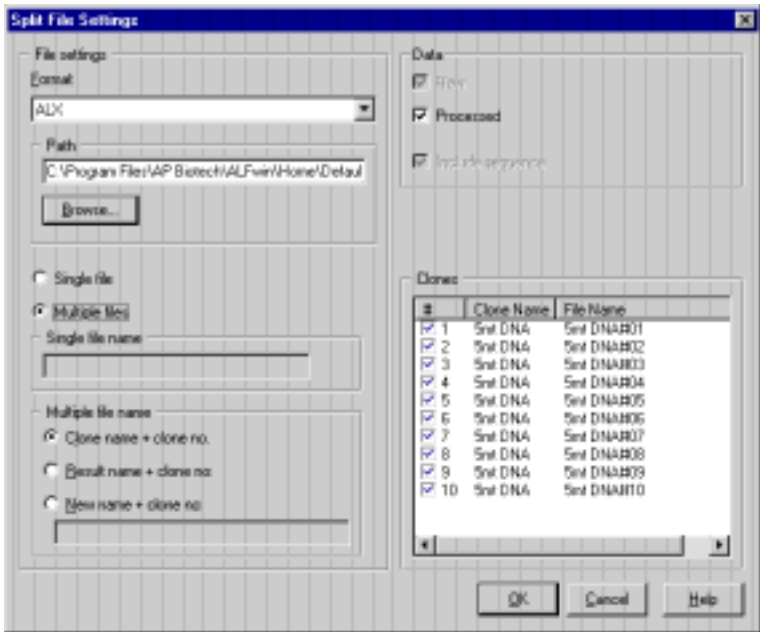


Figure 5-25. Split File Settings dialogue.

2. Select the clones that you want to include in the result file(s).

   If this dialogue is called while the **Curve** or **Gel View** is active, all the clones will be selected by default. If the dialogue is called while the **Result** view is active, only the clones selected in the **Result** view will be selected by default in the **Split File Settings** dialogue.

   Click the tick boxes to de-select or re-select clones.

3. Open the **Format** menu and select the format required. Normally, the .alx format is used. .alf is an older format used in the OS2 version of the program for ALF family instruments.

4. Click on **Browse** and use the **Save As** dialogue to select the destination folder for the file.

5. Select the way in which the clones are saved:

As a **Single file**	Use this option to save the selected clones in one file. Enter the name for the file in the **Single file name** field.
As **Multiple files**	Use this option to save each of the selected clones in a separate file. You must select the naming scheme to be used:
Clone Name + clone no.	Use the name of the clone from the casebook + the clone number.
Result Name + clone no	Use the name of the result file + the clone number.
New Name + clone no.	Use the name typed into the field + the clone number.

6. The raw data for the selected clones is always saved. Activate the **Processed data** button to include processed data.

7. Select the **Include sequence** button if you want to include the base sequence(s) in the saved data. If processed data is selected, the sequences will always be saved.

8. Click on **OK** to save the file(s).

5.11 Exporting data

5.11.1 Exporting curves

You can export curve graphics in a standard Metafile format that can be imported by a number of applications on PC and Macintosh computers. This allows you to include curve graphics in reports being prepared in, for example, Microsoft Word on a PC or Macintosh. The Meta File format can also be imported by many standard graphics applications that you can use to edit an image before it is drafted into a document. Curves and sequence data can also be exported in SCF format. Please refer to Section 5.11.2.

To export a curve graphic as a .wmf-file:

1. Click on the **Curve** window to make sure that it is activated.
2. Using the zoom controls and the scroll bars, adjust the curve window so that it displays the part of the curve you require.
3. Select **File:Export to Meta File**. The **Save As** dialogue is shown.
4. Use the dialogue to select the destination folder for the file.
5. Enter a name for the file.
6. Click **Save**. The picture is saved as a .wmf-file.

5.11.2 Exporting sequences

You can export sequences to files so that they can be loaded into other applications that allow further evaluation of the data. Files can be saved in a range of formats appropriate to various sequence analysis applications. They can also be saved in ASCII format that is accepted by many standard spreadsheet and word processing applications.

Note: You can select the files for export in two ways. They can be selected using the **Export Settings** dialogue as described below. Alternatively, you can select the files in the **Result** window and your selection is transferred into the **Export Settings** dialogue.

To export sequences in a result file:

1. Select **File:Export**. The **Export Settings** dialogue is shown.

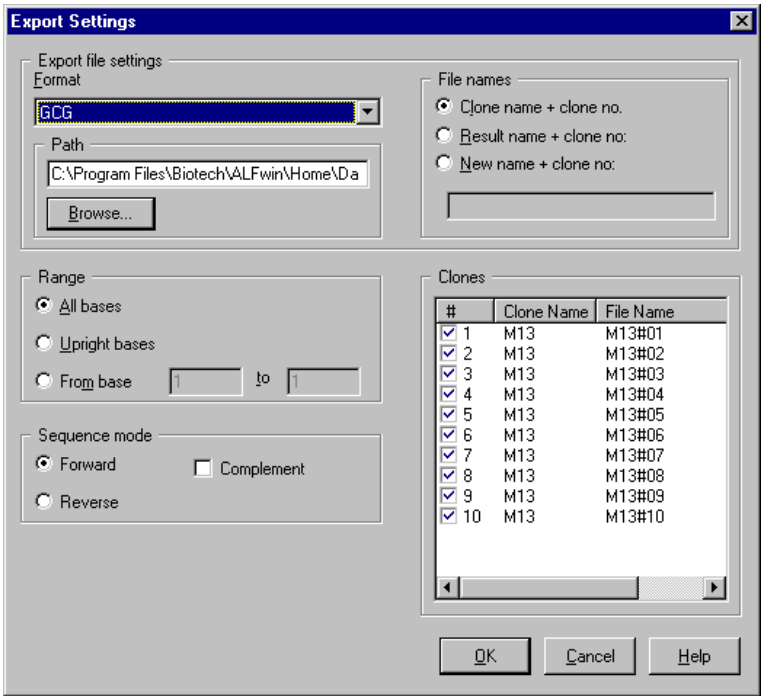


Figure 5-26. Export Settings dialogue.

2. Open the **Format** menu and select the format for the export file:

GCG The .seq format for GCG, the sequence analysis program from Wisconsin University.

STADEN SCF The .scf format for the Staden sequence analysis package, an interactive graphics program for comparing and aligning nucleic acid and amino acid sequences. SCF format includes data curves.

PC/GENE The N prefixed format for PC/GENE, a sequence analysis program by IntelliGenetics from the Oxford Molecular group.

ASCII ASCII .txt format that will load into many standard commercial applications.

EMBL The .eml format for Bioresearch, the sequence analysis program by Fujitsu Ltd.

DNAsis The .dna format of DNAsis, a sequence analysis program from Hitachi Software.

3. If the **Export Settings** dialogue is called while the **Curve** or **Gel View** is active, all the clones will be selected by default. If the dialogue is called while the **Result** view is active, only the clones selected in the **Result** view will be selected by default.
   Click on the tick boxes to select or de-select clones.

4. Enter in the **Path** field the location for the exported file. You can type the path or use **Browse** to open the **Browse** dialogue to select the folder.

5. Select the **Range**:

   All bases All bases in the clones will be exported

   Upright bases Only upright bases in the clones will be exported

   From base...to... Specifies the part of the available clones range to be exported. For example, from base pair number 14 to base pair number 123.

6. Select the **Sequence mode** (this option is not available for Staden SCF format):

   Forward or **Reverse** Export the forward or reverse sequences.

   Complement Export the complement sequence.

7. Select **File names**:

   Clone name + clone no. Use the name of the clone from the casebook + the clone number

   Result name + clone no. Use the name of the result file + the clone number.

   New name + clone no. Use the name typed into the field + the clone number.

8. Click on **OK** to complete the **Export Settings** dialogue.

The sequences are exported to files with the selected format; one file for each sequence.

5.11.3 Copying a clone, a sequence or a Gel View window into another computer application

You can copy clones, sequences and **Gel View** windows so that they can be pasted directly into DTP or graphics applications on the PC. If your computer has a reasonably high resolution screen, a high quality graphic can be produced for a report or ohp transparency.

1. Set the resolution of the computer screen to the maximum possible.

2. Copy the information as follows:

To copy a clone:	click on the **Curve View** window and select **Edit:Copy Clone** or press the <Ctrl+C> keys.
To copy a gel:	click on the **Gel View** window and select **Edit:Copy Gel** or press the <Ctrl+C> keys.
To copy a sequence:	click in the sequence bar or sequence text and select **Edit:Copy Sequence** or press the <Ctrl+C> keys.

3. Open the target application and paste the copied image. This is usually achieved by pressing the <Ctrl+V> keys while the application is active.

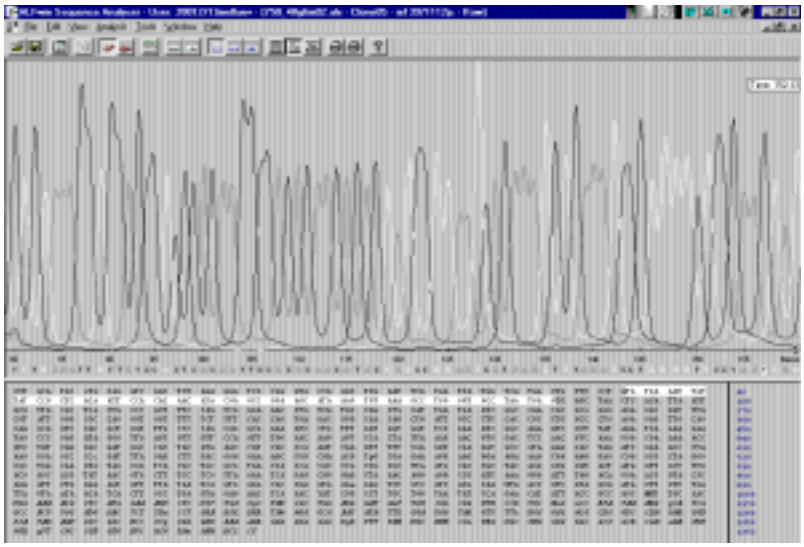


Figure 5-27. Curve window from where clones or sequences can be copied.

5.12 Printing a report

A report is a print that can include items from the casebook, curves, sequences and headers.You select the items you want to include by means of the **Report Settings** dialogue. The settings can be saved as a report layout file that allows you to recall a report configuration. The report layout file does not include information about selected clones. Before printing, the clones must be selected as described below.

1. Select **File:Report**. The **Report Settings** dialogue is shown.

2. If you want to use a report setting that was created and saved in a previous evaluation, click on **Open Layout** and use the **Open** dialogue to select the path and the file name of the layout (.rpt) you wish to use.

   To use the default report layout, open the **Default** folder and select **Report.rpt**.

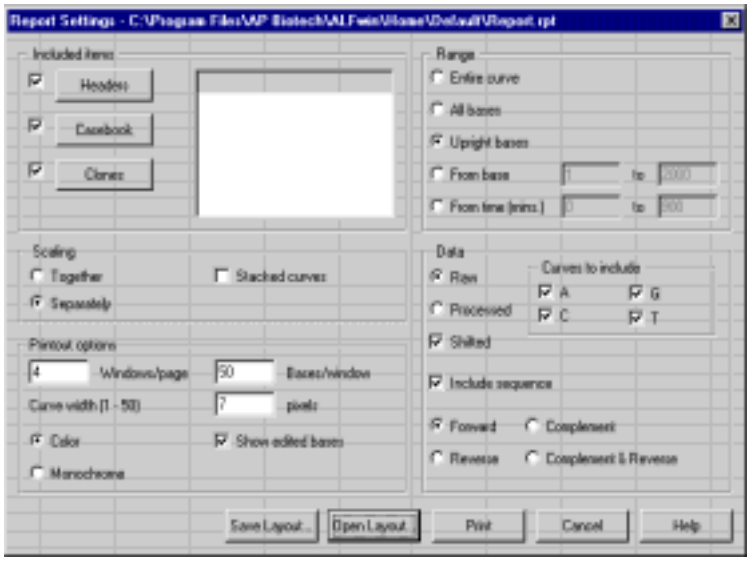


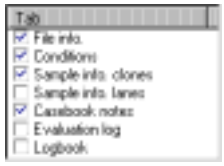
Figure 5-28. Report Settings dialogue.

3. Select the **Headers**, **Casebook** and **Groups** buttons in turn and select items to be included in the report from the lists shown:

Headers

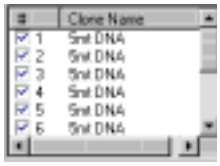
Allows you to select the items that are to be included in the header of the report. You may include any of **Current user**, **Current date** and **File name**.

Casebook



Allows you to select items from the casebook to be included in the report.

Clones



Allows you to select the clones to be included in the report. By default, the clones checked (ticked) in the result window are also checked here. Note that the clone selection is not part of the report layout.

4. Select the **Range** of the curve to be printed:

Entire curve	Curves are printed from the first to the last minute of the run.
All bases	Curves are printed for the called bases.
Upright bases	Curves are printed for the upright bases.
From base...to	Print curves for the bases over the range you specify in the **From...to...** fields.
From time (mins)...to	Print curves for the time period you specify in the **From...to...** fields.

5. Select the type of **Data**:

Raw or **Processed**	Print curves of raw or processed data.
Curves to include	Select the base curves to include in the print.
Shifted	Activate this option to print the curves with the shift applied during processing so that the peaks are spaced at regular intervals.
Include sequence	Activate this option to print the base letters with the curves.
Forward	Print the sequences/curves in forward order.
Reverse	Print the sequences/curves in reversed order.
Complement	Print the complementary sequences/ curves.
Complement & Reverse	Print the complement of the reverse sequences/curves.

6. Select the **Printout options**:

Windows/page	Enter the number of windows to be printed on each page.
Bases/window	Enter the number of bases to be included in each window.
Curve width	Enter the thickness for the curve lines. Range is 1 - 50 pixels.
Colour or Monochrome	The default is colour. Monochrome printouts are useful if you are planning to fax or photocopy the print. Monochrome prints use different line types to differentiate between curves.
Show edited bases	Select this option to underline the edited bases.

7. Select the **Scaling** that is applied to the curves:

Together	Apply the same scaling to all the curves so that only the highest peak uses the whole range of the y-axis.
Separately	Scale each curve to use the whole range of the y-axis.
Stacked curves	Leave this option unchecked to print the curves on one axis. Select the option to print the curves at four different levels in the curve window.

8. If you want to save these settings for later use, click on **Save Layout** and use the **Save As** dialogue to define the path and file name. Click on **Save**.

9. If you want to close the **Report Settings** dialogue without printing a report, click on **Cancel**.

10. Click on **Print** to send the report to the printer.

The next time the same user opens the **Report Settings** dialogue, the settings will be the same as in the last saved and used setup.

5.13 Printing Curve and Gel Views and sequences

5.13.1 Printing the contents of a single view

The **Print** command allows you to print the contents of the active view. By this means you can obtain a print of a **Curve** or **Gel View** or a sequence.

1. Click on the view required to ensure that it is activated.

2. Adjust the window size and contents to display the region that you want to print.

3. If you want to check that the print is appropriate to your requirements, select **File:Print Preview**. A preview of the printed document is shown on screen.

   Use the buttons to adjust the view:

Next page and **Previous page**	to navigate a multipage table (not applicable in this instance).
One Page/**Two Page**	to adjust the number of table pages displayed (not applicable in this instance).
Zoom in and **Zoom out**	to adjust the magnification of the print image.

4. To print from the print preview, click on the **Print** button. Otherwise, click on the **Close** button to close the preview.

5. To print from a selected window, select **File:Print**. The **Print** dialogue opens. Use the dialogue according to standard Windows 95 conventions.

5.13.2 Printing all views

The **Print All** command allows you to print all the open **Curve** windows.

1. Open the **Curve** window(s) and make adjustments so that they display the region you want to print.

2. If you want to check that the print is appropriate to your requirements, select **File:Print All Preview**. A preview of the printed document is shown on screen.

   Use the buttons to adjust the view:

Next page and **Previous page**	to navigate a multipage document.
One Page/**Two Page**	to adjust the number of pages displayed.
Zoom in and **Zoom out**	to adjust the image magnification.

6. To print from the print preview, click on the **Print** button. Otherwise, click on the **Close** button to close the preview.

7. To print from a selected window, select **File:Print All**. The **Print** dialogue opens. Use the dialogue according to standard Windows 95 conventions.

5.14 Using tools

Tools are shortcuts to other programs. Add shortcuts to programs that you often use in connection with ALFwin Sequence Analyser, for example, Microsoft Excel or Word, HLA Sequityper, Mutation Analyser etc. Use tools to open these programs directly from ALFwin Sequence Analyser.

The tools supplied with ALFwin Sequence Analyser

ALFwin Sequence Analyser is supplied with two tools:

Convert ALX/ALF to TIFF	Tagged Image File Format is a commonly used bitmap format that allows graphical images to be imported into other applications such as Image Master.
Decompress ALF File	ALFwin Sequence Analyser accepts .alx and .alf file formats. The .alf format preceeded the present .alx format. If the .alf files have been saved in compressed format, they must be decompressed before they are opened by ALFwin Sequence Analyser.

Viewing settings for the supplied tools

The **Tools** dialogue allows you to define up to six tools that can be launched directly from the **Tools** menu in ALFwin Sequence Analyser. To open the Tools dialogue, select **Tools:Edit Tools**.

If you want to view the default settings, open the **Items** list and select the appropriate tool. Information is presented in the **Tools** dialogue for:

Command	This is the command line to launch the tool.
Argument	This shows any extra arguments relating to the command line.
Initial Directory	This is the folder that contains other important components needed by the tool.

Editing the settings for the supplied tools

1. Open the **Items** list and select the appropriate tool. Click on the **Edit Tool** button and the **Edit Tool** dialogue is displayed.

2. Edit the appropriate information:

Command	This is the command line to launch the tool.
Argument	This shows any extra arguments relating to the command line.
Initial Directory	This is the folder that contains other important components needed by the tool.

3. Click on **Save Tool**.

Adding new tools

You can define new tools (programs)which can be launched from within ALFwin Sequence Analyser.

Note: You can only define a maximum of six tools including the two tools supplied with ALFwin Sequence Analyser.

1. Click on the **Add Tool** button and the **Define Tool** dialogue is displayed.

2. Enter the appropriate information:

Name	Enter a name for the tool.
Command	Enter the command line to launch the tool. Use the Browse facility to help you.
Argument	Enter any extra arguments relating to the command line.
Initial Directory	Enter the folder that contains other important components needed by the tool. Use the Browse facility to help you.

Deleting tools

To delete a tool:

1. Open the **Items** list and select the appropriate tool.

2. Click on the **Delete Tool** button.

5.14.1 The .alx and .alf formats

ALFwin Sequence Analyser v.2.00 operates in the main with the .alx file format. ALFwin Sequence Analyser can also use files of the earlier .alf type.

There are two reasons why you may wish to use the .alf format in your work:

- You have data which were collected and saved before .alx filetype became standard.
- You have computer applications which are designed to operate on files in .alf format.

In the latter case, you may wish to save data from ALFwin Sequence Analyser in .alf format. If you use the normal **File:Save** option to save your result file, ALFwin Sequence Analyser will save it in .alx format. To save as an .alf file you must use the **Split File Settings** dialogue. Please refer to Section 5.10.2.

5.14.2 Decompressing .alf files

ALF files generated in earlier versions of ALF family instrument control software, e.g. ALF Manager and AM, could save ALF files in a compressed format. To decompress an ALF file:

1. Select **File:Open** and proceed using the normal file opening procedure. Please refer to Section 5.3.2.

2. To open a decompressed ALF file, click on **Open** and locate the appropriate file in the displayed dialogue. Click on **Open** to return to the previous dialogue. The name of the file is displayed in the **Compressed file** field.

3. The destination path and file name should be automatically displayed if the **Tools:Add file** command is checked. If required, alter the destination path and folder for the file after decompression. Use the **Browse** option to define the path using the standard Windows 95 dialogues.

4. Click on **OK** to decompress and open the file.

5.14.3 Converting ALX/ALF files to TIFF

You can convert ALF or ALX files into a Tagged Image File Format (TIFF) that can be imported as a graphic into other software applications. This will be viewed as a fluorogram (gel).

1. Select **Tools:Convert ALX/ALF to TIFF**. The **Convert ALX/ALF to TIFF** dialogue is displayed.

2. To open an ALX or ALF file for conversion, click on **Open** and locate the appropriate file. Click on **Open** to return to the **Convert ALX/ALF to TIFF** dialogue for the opened file.

3. Select the **Lanes** that will be included in the TIFF file. By default, all are selected. Specific lanes can be selected by holding down the <Ctrl> key.

4. Alter any other information as required:

   Start time/Stop time (sec) These fields allow you to change the default range of the sequencing data included in the TIFF file based on the start and stop times that you enter.

Resample — This allows you to set the sampling occasion of data points for generation of the TIFF file. A larger value uses fewer data points, i.e. data points are taken at every 'x' interval. Use of few data points reduces the size of the generated file, although also reduces the resolution of the image.

Data Type — In some cases, you can generate a TIFF file based on the raw data or processed data.

Gutter — This is the number of pixels separating the lanes in the image.

Lane width — This is the width in pixels of each lane in the image.

Resolution — In some cases, you can select an alternative resolution for the TIFF file.

Compression — In some cases, you can select a compression tool for the generated TIFF file.

Invert option box — This option produces the TIFF file as a negative image.

Image width/height — These closed fields show the width and height in pixels of the image to be generated based on the selections you make in other fields.

5. To execute the conversion, click on the **Convert** button. The generated TIFF file is stored in the folder defined in the **Initial Directory** field of the **Tools** dialogue.

5.14.4 Add file

The **Tools:Add file** command, when active (checked), can for some programs automatically display the pathway and file name of the target file based on the path and name of the source file. Only the file extension is changed.

5.15 Exiting ALFwin Sequence Analyser

When you have finished working with the result file, you can close ALFwin Sequence Analyser:

Select **File:Exit** or press the <Alt+F4> keys or click the **Close** button (in the upper right corner of the window).Alternatively, if you are going to use ALFwin Sequence Analyser again soon, you can temporarily hide the
ALFwin - Sequence Analyser window by using the minimize button (in the upper right corner of the window).

6 Sequence Alignment

ALFwin Sequence Analyser contains an integral module for alignment of sample sequences. Sample sequences for alignment can be obtained from a combination of sources:

- by opening a result file in ALFwin Sequence Analyser and processing the raw data to produce a sequence for each clone
- by importing sequence data in other formats.

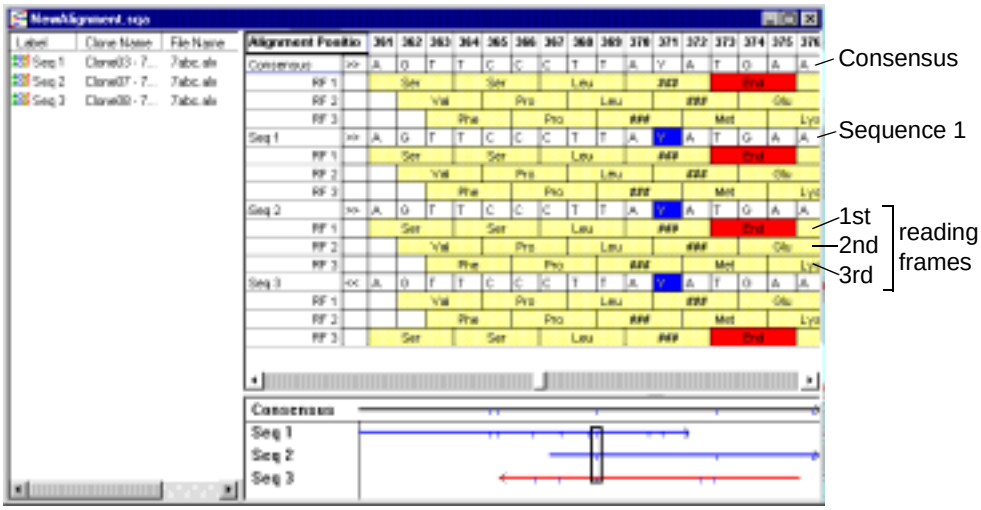


Figure 6-1. A typical alignment item, including the sequences and the alignment table showing the aligned bases with the amino acid reading frames added. The alignment overview indicates the area displayed in the alignment table.

Sequence alignment compares sample sequences from within the same result file or from different runs. When the sequence alignment is calculated, all sample sequences are aligned with each other and the best alignment shown. A consensus sequence is also generated that represents the combined alignment between the sample sequences, which allows base positions with imperfect alignment to be easily identified and, if necessary, edited. The full graphical presentation provides you with a direct link to sequence curves and statistical data, giving you a powerful qualitative and quantitative analysis tool for sequence alignment.

Key features include:

Alignment of areas of similarity between sequences	Sample sequences are compared in the forward and complementary-reverse directions and the best alignment is displayed. This is useful for comparing similar sequence data from the same or different runs or locating overlapping regions between neighbouring sequencing fragments
Automated generation of a consensus sequence	This is generated from the consensus of the aligned sample sequences, which allows you to view the overall result.
Location of mismatching sequence base positions	Sequence base mismatch positions between aligned sample sequences are automatically located and colour coded. This is useful for comparing similar sequence data from the same or different runs and editing the sequence bases.
Location of sequence gaps	Gaps in the aligned sequences are easily identified in the sample sequences and consensus sequence.
Generation of amino acid sequences	These can be generated for each of the aligned sequences and you can generate amino acid sequences for the collective data in any or all of the three possible reading frames.
Alignment export facilities	Selected sample sequences and all aligned sequences can be exported in MSF[a] or PHYLIP[b] format. The consensus or the amino acid sequences can be exported in ASCII format.
Statistical summary	The statistical function provides a quantitative measurement of the quality of the alignment.

Alignment of sequences from different sources	Sequences can be included for alignment either as a clone sequence from ALFwin Sequence Analyser, as imported sequences in ASCII format, and as imported sequences in SCF format.
Interactive editing features	The alignment table display allows you to quickly jump to selected parts of the sequences where you can examine the bases in each sequence and view the related parts of the raw or processed data curves. Full editing facilities allow you to make changes on the basis of this inspection. Any changes you make are documented automatically in the alignment log and there is a note pad where you can write your own notes.

a. MSF is the multiple sequence alignment format of the GCG sequence analysis package. Information can be found at: http://www.embl-heidelberg.de/predictprotein/new/Dexa/optin_msfDes.html
b. PHYLIP (the PHYLogeny Inference Package) is a package of programs for inferring phylogenies (evolutionary trees). Information can be found at: http://evolution.genetics.washington.edu/phylip.html

A typical session with sequence alignment involves:

- opening the appropriate result file(s), processing the sequences and/or importing sequences in other formats
- creating a new sequence alignment item
- aligning the sequences
- viewing and editing the sequences
- generating the amino acid sequence in the desired reading frame
- saving and/or exporting the results
- printing a report.

This chapter is divided into the following sections:

6.1 Creating a sequence alignment item
6.2 Aligning the sequences
6.3 The alignment overview
6.4 The alignment table
6.5 The consensus sequence
6.6 Sequence Alignment statistics
6.7 Editing sequence bases from the sequence alignment
6.8 Amino acid sequences
6.9 The alignment log
6.10 The alignment notes
6.11 Saving the sequence alignment
6.12 Printing an alignment report
6.13 Exporting sequence alignment data
6.14 Opening a previously saved sequence alignment item
6.15 Configuring the sequence alignment module

6.1 Creating a sequence alignment item

Sequence data from several sources can be used for sequence alignment. You can use:

- sequences from ALFwin data files obtained from processing raw data
- sequences imported into ALFwin Sequence Analyser in ASCII format
- sequences imported into ALFwin Sequence Analyser in STADEN SCF format
- a combination of the above.

You should open all of the result files containing sequence data for the clones and also import any other sequences, before you create a sequence alignment item. All sample sequences from open **Result** windows in ALFwin Sequence Analyser are then automatically added to a new alignment item.

Note: Raw data without sequences cannot be used in sequence alignment. The raw data must first be processed in ALFwin Sequence Analyser.

6.1.1 Creating a new sequence alignment item

Select **File:New Alignment Item**. The **Alignment Item** window is shown containing a list of all sequences from the open **Result** windows.

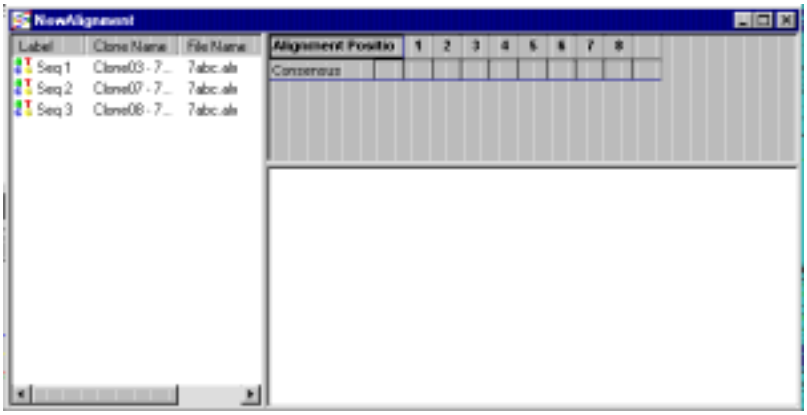


Figure 6-2. Alignment Item window.

The sequences are displayed in a sequence alignment list.

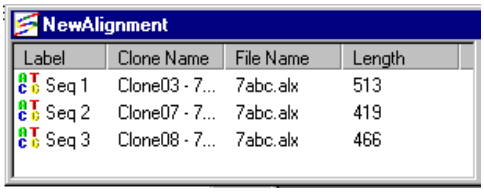


Figure 6-3. Sequence alignment list as one part in the Alignment Item window containing sequences for fragment 3 of the p53 gene.

Various information is presented for each sequence, which includes:

Icon — The icon to the left of each sequence gives its status in Sequence Alignment. In the following table, examples are given for the type of effect seen on an icon dependent on its status. An icon can have more than one of the effects described in the right column and the icon will change according to changing status.

Default icon and status	*Effect on icon and status*
Non-aligned sequence.	The sequence has been aligned.
Source **Result** file for the sequence open in ALFwin Sequence Analyser.	Top right edge folded over; Source **Result** file for the sequence not open in ALFwin Sequence Analyser, i.e. a saved alignment item has been opened but not the source Result file.
Raw data for the sequence is available.	Grey background to icon; Raw data for the sequence is unavailable, which usually indicates an imported sequence format. This means that raw data curves cannot be viewed for this sequence
Sequence is imported into Sequence Alignment.	Red background to icon; Sequence has been edited in ALFwin Sequence Analyser but not re-imported into Sequence Alignment.
Source file for the sequence in its original folder.	Greyed icon; Source file for the sequence is not in its original folder. This usually occurs when an alignment file is moved between computers.

Label Each sequence is given a numbered sequence label, e.g. **Seq1**, **Seq2** etc. The sequence numbers allow sequences with the same name to be included in the sequence alignment item. This is useful when sequences with the same name are selected from different result files. These **Labels** can be edited by clicking on the name to highlight it and typing in the new name.

Clone Name This is the 'clone' name and number for the sequence as displayed in the source **Result** window. Note that sequences imported from other formats are placed into a **Result** window and are given a clone name and number.

File Name This displays the source file name for the sequence.

Length This shows the length of the sequence.

6.1.2 Adding further sequences before alignment

If you want to add further sequences to the sequence alignment list before performing an alignment:

1. Open the result files or import the files containing additional sequences.

2. Select each newly opened **Result** window in turn and select the sequences to be added. Select **Analysis:Add To Alignment**. Sequences are added to the sequence alignment list in the **Alignment Item** window.

6.1.3 Changing the order of sequences

To change the order of the sequences in the sequence alignment list, click a sequence name in the sequence alignment list and drag it to the required position.

6.1.4 Removing sequences

To remove sequences from the sequence alignment list:

1. Select the sequence(s) from the list that you want to remove. Press the <Ctrl> key to select several sequences.

2. Select **Edit:Remove sequences**. The sequences are removed from the list.

6.2 Aligning the sequences

This section describes how to perform an alignment.

6.2.1 Aligning all sample sequences

The usual way to perform an alignment is to create a new alignment item containing all of the sequences that you want to align and then use the **Analysis:Align All** function. This function aligns all sequences with respect to one another, testing the alignment in the forward direction, and also the reverse complementary direction. As a result, the best alignment of the sample sequences is displayed.

A consensus sequence is also produced (see Section 6.5), which is a representative sequence for all aligned sample sequences. The consensus sequence and aligned sample sequences are linked. If you edit a base position in a sample sequence, the corresponding position in the consensus sequence will change in accordance with the edit. Conversely, if you edit a base in the consensus sequence, the corresponding base position in all aligned sample sequences will change to be the same as in the consensus sequence.

To initiate the alignment process:

1. Click on the **Alignment Item** window to ensure that it is active.

2. Select **Analysis:Align All** or click on the **Align All** button.

   The results are shown as an alignment overview (see Section 6.3) and as an alignment table (see Section 6.4).

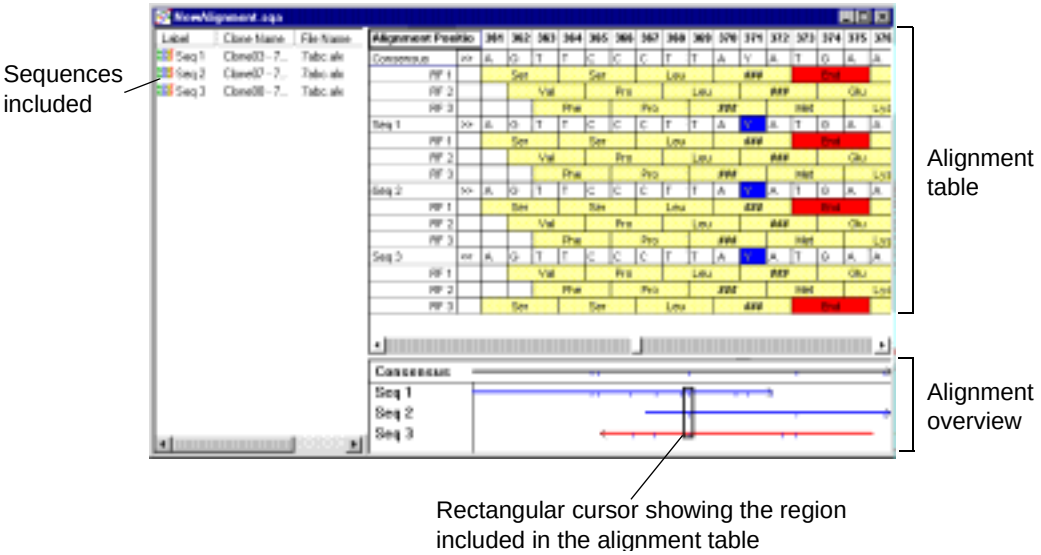

Sequences included
Alignment table
Alignment overview
Rectangular cursor showing the region included in the alignment table

Figure 6-4. Alignment Item window after alignment of all sequences.

6.2.2 Aligning additional sequences to the previous alignment

After you have performed an alignment, you may later wish to add more sequences to the sequence alignment item and add them to the previous alignment. This is achieved using the **Analysis:Align to Previous** function whereby the new sequence(s) are aligned with the consensus sequence that was generated in the previous alignment, i.e. there is no new alignment involving all of the existing sequences, which contrasts with the **Align All** function.

The consensus sequence is automatically updated to reflect the alignment with respect to the *new* sequence(s). If as a result of the **Align to Previous** function the consensus sequence is changed in any way, this will in turn be reflected in the other sample sequences used in the original alignment. You may also at any time perform a full alignment by selecting **Analysis:Align All** whereby all of the sample sequences in the sequence alignment list are aligned and contribute to the generation of a new consensus sequence.

You can align additional sequences to the previous alignment.

1. Open the result files or import other sequence files.

2. Select each newly opened **Result** window in turn and select the sequences to be added. Select **Analysis:Add To Alignment**. Sequences are added to the sequence alignment list in the **Alignment Item** window.

3. Select the sequences that are to be aligned to the previous alignment. Press the <Ctrl> key to select several sequences.

4. Select **Analysis:Align to Previous** or click on the **Align Sequence** button. The results are shown in the alignment overview (see Section 6.3) and alignment table (see Section 6.4).

6.3 The alignment overview

Results of an alignment are displayed in the alignment overview and alignment table (see Section 6.4).

The alignment overview shows a graphical representation of the whole result. Each sequence is displayed as a line and the length corresponds to the number of bases in the sequence. All lines are positioned relative to one another based on the results of the alignment. The alignment overview includes a rectangular cursor that indicates the part of the result currently displayed in the alignment table. You use this cursor to navigate around the sequences.

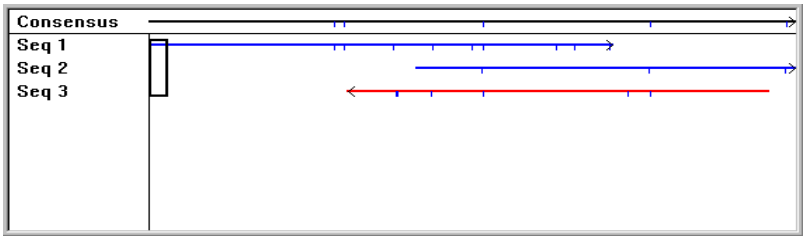


Figure 6-5. Alignment overview in the Alignment Item window.

6.3.1 Sample sequence lines and alignment direction

A blue sample sequence line going from left to right, as denoted by the direction of the arrow head, indicates a sequence that is aligned in the forward direction. A red sample sequence line going from right to left, as denoted by the direction of the arrow head, indicates a sequence that

is aligned in its complementary-reverse direction.

The default settings for the alignment calculation determines the best direction of the sequences for alignment purposes. You can manually change the direction of all sequences by selecting (checking) **Edit:Rev + Comp Sequences**. This reverses the direction of all displayed sequences. This is especially useful in determining the correct sequencing direction with respect to the amino acid sequence (see Section 6.8).

Original alignment display	*Complementary and Reverse alignment display*
Blue line for sequences in the forward direction, becomes:	red line in complementary-reverse direction
Red line for sequences in complementary-reverse direction, becomes:	blue line for sequences in the forward direction

6.3.2 Consensus line

A black consensus sequence line is displayed above the sample sequence lines. The consensus sequence is automatically generated during the alignment process and is a representative sequence of all aligned sample sequences. Further details are given in Section 6.5.

6.3.3 Rectangular cursor

A rectangular cursor spans several or all of the sequence lines corresponding to the visible number of sequences in the alignment table above. The position and width of the rectangular cursor relates to the area of the spanned sequences for which specific alignment details are shown in the alignment table. Use the mouse pointer to drag the cursor rectangle to any desired position to display a new view in the alignment table or double click on the desired portion of the alignment overview.

6.3.4 Alignment imperfections

Imperfections in the matching of specific base positions between aligned sequences, i.e. detected single base components, are indicated by short vertical tick marks on the sample sequence lines. The tick marks are colour coded to indicate the type of imperfection.

Note: The colour codes can be re-defined in the **Alignment Properties** dialogue (see Section 6.15.5).

No tick mark	All the sample sequences that extend to that region have the same single base component.
Yellow tick mark	There is a gap at this position.
Blue tick mark	There is an ambiguity at this position.
Red tick mark	There is a single base component mismatch at this position.

Tick marks displayed on the consensus sequence are also dependent on the consensus mode being used (see Section 6.5.1).

Use the mouse to drag the rectangular cursor to a position that encompasses the tick marks you want to evaluate. The alignment table changes to show the region of the base sequences covered by the cursor.

6.4 The alignment table

The alignment table displays the actual sequence data for each of the aligned sample sequences and the consensus sequence.

The area of the aligned sequences shown corresponds to the position of the rectangular cursor in the alignment overview (see Section 6.3.3). You can view different areas of the alignment by moving the rectangular cursor or by using the horizontal scroll bar beneath the alignment table.

Alignment Position		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	1
Consensus	>>	g	a	G	T	A	T	T	T	G	G	c	A	T	G	c	A	C	A	G
Seq 1	>>			G	T	A	T	T	t	G	G	-	A	T	G	-	A	C	A	G
Seq 2	>>			G	T	A	T	T	T	G	G	-	A	T	G	-	A	C	A	G
Seq 3	>>	G	A	G	T	A	T	T	T	G	G	-	A	T	G	-	A	C	A	G
Seq 4	>>			G	T	A	T	T	T	G	G	C	A	T	G	C	A	C	A	G
Seq 5	>>	G	A	G	T	A	T	T	T	G	G	-	A	T	G	-	A	C	A	G

Figure 6-6. Alignment table in the Alignment Item window.

6.4.1 Sample sequences

The sample sequences are presented in tabular form. Each sample sequence forms a row in the table and the individual bases comprising the sequence are displayed in individual cells within the sequence row. The sequence rows are positioned relative to one another based on the calculated alignment so that corresponding base positions are displayed in the same column.

6.4.2 Consensus sequence

The consensus sequence is displayed in a row above the sample sequence rows. The consensus sequence is automatically generated during the alignment process and is a representative sequence of all sequence data. Further details are described in Section 6.5.

6.4.3 Alignment direction

The alignment direction of individual sequences is denoted by the symbol **>>** for forward and **<<** for complemetary reversed.

6.4.4 Alignment imperfections

Imperfections in the matching of specific base positions between aligned sequences with respect to the consensus sequence, i.e. detected single base components, are indicated by colour coded cells. These correspond to the colour coded ticks on the sequence lines in the alignment overview. The colour codes can be re-defined in the **Alignment Properties** dialogue (see Section 6.15.5).

White cell	All sample sequences extending to that region have the same base component.
Yellow cell	There is a gap at this position.
Blue cell	There is an ambiguity at this position.
Red cell	There is a single base component mismatch at this position compared to the consensus.

6.4.5 Adjusting the alignment table

When all the required data is displayed, you may wish to adjust the rows and columns in the table:

To adjust the height of a row of cells	Move the mouse pointer into the grey sequence column and position it on the horizontal border you want to move. The mouse pointer becomes an up/down arrow. Click and drag the border to the new position.
To adjust the width of a column of cells	Move the mouse pointer into the grey header column and position it on the vertical border you want to move. The mouse pointer becomes an left/right arrow. Click and drag the border to the new position.
To display or hide the gridlines	Use **View:User Preferences** to select or deselect **Display Gridlines**.

6.5 The consensus sequence

The consensus sequence is a product of the alignment process, which gives you an overall result representing the contribution from all the sequences. This allows you to easily assess the quality of alignment.

The consensus sequence is automatically generated after all of the sample sequences have been aligned. The algorithm achieves this by comparing the base components between the aligned sequences at each base position. This produces a consensus base for each position that is used to build up the consensus sequence. It is possible to select different modes for calculating the consensus, and these will affect the content of the consensus sequence.

6.5.1 The consensus modes

The consensus sequence can be configured in either of two modes:

Least Common Denominator	This is the default mode and uses the simplest IUPAC base code which incorporates all the contributions from the sequences. A small letter is used if any of the contributions are blanks or if any of the sequences have no contribution.

An ambiguity in any of the sequence bases is conferred on the consensus. Least common denominator mode is illustrated by the following example:

Consensus	A	g	R	Y	B	v
Seq 1	A	G	A	Y	Y	A
Seq 2	A	-	G	Y	K	v
Seq 3	A	G	G	Y	Y	A

B = CTG K = GT R = AG
V = ACG Y = CT

Majority

Consensus uses the base code letter which appears most frequently in the sequences. If there is no letter in majority, the appropriate ambiguity code is used. Gaps are ignored unless they occur in a majority in which case the remaining base letters are represented by a lowercase letter in the consensus. Majority mode is illustrated by the following example:

Consensus	G	C	R	K	a	Y
Seq 1	G	C	A	K	A	C
Seq 2	G	A	A	G	A	T
Seq 3	G	C	G	K	-	-
Seq 4	G	C	G	K	-	-
Seq 5	G	A	-	G	-	-

(R = AG K = GT Y = CT)

Changing the consensus mode

The following procedure is used to configure the consensus mode. These settings will hold between sessions for each user. If you want to alter the default (startup) configuration, refer to Section 6.15.1.

1. From the **ALFwin Sequence Alignment** dialogue, select **View:Consensus**.

2. Select **Least Common Denominator** or **Majority** according to your requirements.

6.5.2 Recalculating the consensus sequence

By default, the consensus is automatically recalculated when you edit bases in the sample sequences within the alignment table or change the consensus mode.

To disable automatic recalculation of consensus	Select **View:User Preferences** and click **Automatic Recalculation of Consensus** to deselect it.
To initiate a recalculation manually	When automatic recalculation of consensus is disabled, recalculate by selecting **View:Consensus:Recalculate Consensus**.
To restore automatic recalculation	Select **View:User Preferences** and click on **Automatic Recalculation of Consensus** to select it**.**

6.5.3 Viewing the IUPAC codes

If you locate an ambiguity code in the consensus sequence, you can display the IUPAC codes.

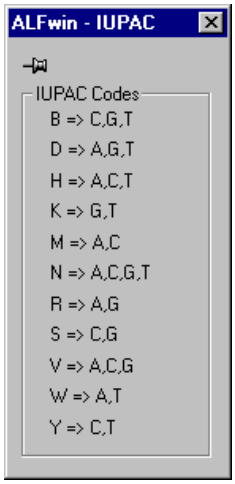


Figure 6-7. IUPAC dialogue.

- The **IUPAC** dialogue is automatically displayed when you click on an ambiguity code in the alignment table.
- To keep the dialogue active on the desktop after you have selected another window, click on the pin-button icon. The icon is depressed.
- To cancel the dialogue, click on the pin-button icon again so that it pops out. Select another window and the dialogue automatically disappears. Alternatively, click on the dialogue close button in the top left corner.
- The automatic display of IUPAC codes when an ambiguity is selected in the alignment table can be suppressed by selecting (unchecking) **View:User Preferences:Auto Display of Ambiguity Popup Table**.
- If the dialogue is hidden from view select **View:IUPAC Codes**.

6.6 Sequence alignment statistics

The sequence alignment module provides a statistical overview which you use to assess the quality of the alignment. The statistics allow a quantitative assessment of the initial alignment and can be used to measure the effect of any adjustment you make to the data.

6.6.1 Displaying the alignment statistics

The statistics overview is displayed automatically when alignment processing is completed.

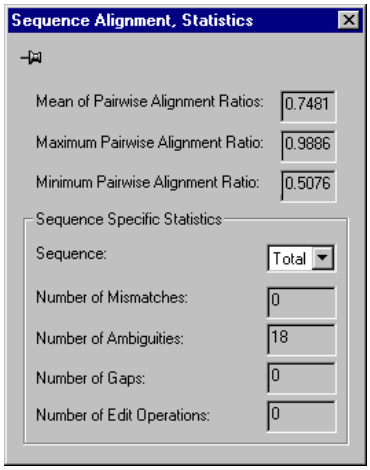


Figure 6-8. Sequence Alignment, Statistics dialogue.

To make the statistics window stay on screen as the focus is shifted to another window, click on the pin-button icon in the top left corner of the dialogue. The pin-button icon then appears depressed. To remove the effect of pinning, click on the pin-button icon again so that it appears to pop out, or else click on the dialogue close button in the top right corner. You can switch the auto display function on and off by selecting **View:User Preferences** and selecting or deselecting **Auto Display of Statistics Popup Table**.

To again display the alignment statistics, select **View:Alignment Statistics**. The **ALFwin Sequence Alignment, Statistics** dialogue opens.

6.6.2 Explaining the alignment statistics

The **Sequence Alignment, Statistics** dialogue can be used to view statistics for the whole alignment or for a specifically selected sequence. This is determined in the **Sequence** drop-down list, which by default shows **Total** to indicate that the displayed statistics are for all sample sequences. To view the statistics for a specific sequence, click on the drop-down list and select a specific sequence.

Alignment quality

An overall measure of the quality of the alignment is also provided through the three parameters, **Mean of Pairwise Alignment Ratios**, **Maximum Pairwise Alignment Ratio** and **Minimum Pairwise Alignment Ratio**. A pairwise alignment ratio is calculated in the following manner:

The maximum total score value from the forward and complement-reverse alignments is divided by the smallest length of the two sequences included, multiplied by the match score value.

$$\text{Alignment Ratio} = \frac{\text{Maximum total score}}{\text{Minimum length} * \text{Match score}}$$

Where:

Total score	is the resulting alignment score obtained by aligning two sequences according to the Needleman Wunsch algorithm.
Maximum total score	is the maximum score from forward and reverse-complement alignment of two sequences.
Minimum length	is the length of the shortest sequence in an alignment of two sequences.

Match score	is the score assigned to a single match of identical bases in two aligned sequences.

This is a crude measure, but generally gives an overall quality measure of the alignment. The optimal **Mean of Pairwise Alignment Ratio** is 1, which is obtained if both aligned sequences are identical. Therefore, the closer the mean value is to 1, the better the quality of the alignment.

If the **Minimum** and **Maximum Pairwise Alignment Ratios** are not close, it indicates a lower degree of similarity between the sequences, especially if the minimum ratio is very small. In such a case, it is advisable to remove the sequence(s) that appear not to align well with the others. Conversely, if the minimum and maximum ratios are close, all of the included sequences show a high degree of similarity.

It is not possible to get the specific alignment ratios between any two sequences, since most of the alignment operations are actually performed on virtual sequences, which are sequences that make up a consensus between two or more other sequences. These intermediate consensus sequences are not registered anywhere. Only the final alignment and the final consensus are listed.

This quality measure should not be subject to any deeper study of the alignment, since it is quite crude. It gives some clues as to whether the sequences are similar or not and it can also be useful when trying out different algorithm settings. The setting that gives the highest mean alignment ratio is, in most cases, the setting that gives the best final alignment.

Sequence-specific statistics

The statistics give the following information about alignment imperfections:

Number of mismatches	This is the number of resolved base component mismatches in the sample sequence(s).
Number of Ambiguities	This is the number of unresolved base component mismatches (ambiguities) in the sample sequence(s).
Number of gaps	This is the number of gaps in the sequence(s).
Number of edit operations	This is the number of base positions that have been edited in the sequence(s) of the alignment

6.7 Editing sequence bases from sequence alignment

It is possible to edit individual bases in all of the sample sequences and also in the consensus sequence, to improve the overall quality of the data. This is achieved by editing the bases directly in the alignment table. Provided that a selected sequence originates from an ALFwin ALX/ALF data file, a powerful feature of the sequence alignment module allows you to select a sample sequence base position in the alignment table and view the corresponding curves in the **Curve** window of ALFwin Sequence Analyser.

Note: Edit operations that you perform in Sequence Alignment do not affect the original sequence data. If you want to edit the original data, you must do this in the **Curve** window of ALFwin Sequence Analyser.

6.7.1 Editing a base position

The outcome of editing a base position depends on whether the change is made to any of the included sample sequences or to the consensus sequence. Moreover, the displayed result for the consensus sequence resulting from an edit operation is dependent on the selected consensus mode (see Section 6.5.1).

When you change a base in a sample sequence and the consensus sequence is recalculated, the change is reflected in the consensus sequence. The edited base is underlined. When a base is changed in a consensus sequence, the bases at that position in all the sequences are changed to validate the consensus. All the sample sequence bases at that position become underlined letters but the consensus base you edited is not underlined.

To replace a base.	To replace a base, first activate the Replace mode (selected by default) by selecting **Edit:Replace** or clicking on the **Replace Mode** button. Click on the letter of the base that you wish to replace and type the alternative letter you want at that position. Replace mode is the default editing mode indicated by the check mark beside the **Edit:Replace** option. Replace mode toggles with the Insert mode.

To insert a base (sample sequences only)	To insert a base, first activate the Insert mode by selecting **Edit:Insert** or clicking on the **Insert Mode** button.
	Select the base to the right of the insert position. Type the letter you want to insert and the base is inserted. All bases to the right are shifted one position. An active Insert mode is indicated by the check mark beside the **Edit:Insert** option. Insert mode toggles with the Replace mode.
To delete a base	Click on the base that you want to delete. Select **Edit:Delete or** press the <Del> key. Deletions are emphasised by a bold left border to the cell from which the base was deleted.
To undo the last edit action	Select **Edit:Undo** or press the <Ctrl + Z> keys. Only the last edit operation can be undone.

Note: By default, you will receive a warning when you edit a base in the consensus sequence. If you do not want to see this warning, deselect (uncheck) the **View:User Preferences: Warn on Consensus Editing** command.

6.7.2 Shifting sequences

It is possible to shift one or several sequences in the alignment table to the left or right of the current position.

1. Select the sequences that you want to shift in the alignment table.

2. To shift the selected sequences one base position to the left, select **Edit:Shift Left** or click on the **Shift Left** button. Conversely, to shift the selected sequences one base position to the right, select **Edit:Shift Right** or click on the **Shift Right** button.

If the alignment is affected by the shift, the consensus sequence is automatically recalculated to reflect the new alignment.

Note: Sequences cannot be shifted in any direction that would cause the extreme base positions to be lost from the alignment table.

6.7.3 Viewing and editing bases from Sequence Alignment using the Curve window

Provided that a selected sequence originates from an ALFwin ALX/ ALF data file, you can move directly from a base in the alignment table and view its position in its **Curve** window:

1. From the alignment table, double click the base that you want to investigate, or select the base and click on the **View Curves** button.

2. The raw data **Curve** window for the selected sequence opens with the selected base highlighted.

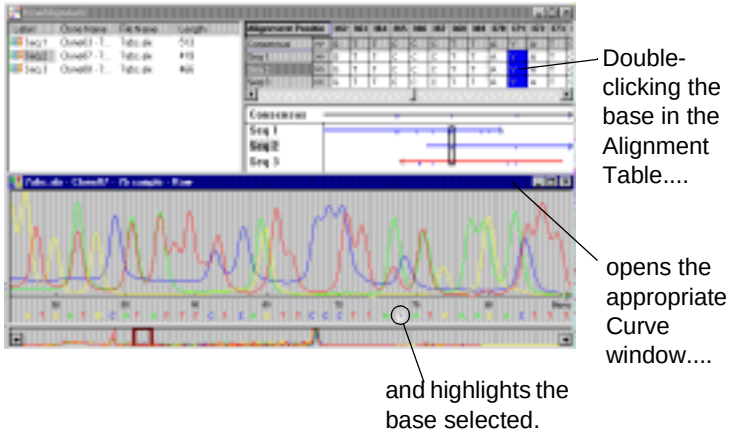


Figure 6-9. Showing how selecting a base in the alignment table opens the appropriate Curve window, locates and highlights the base.

3. Adjust the **Curve** window so that you can obtain a clear view of the curve for the selected base.

4. Open any other curves you need by repeating the above procedure. You can select opened curves for viewing by opening the **Window** menu and selecting the curve name from the list. Alternatively, use **Window:Tile All Curve Windows** to compare the curves.

5. Edit any bases in the **Curve** view as described in Section 5.8.

6. Click on the alignment item. If you have edited any of the original data, you will receive a message asking if you want to re-import the edited sequence. Click on **OK** to re-import the sequence. A second message is then displayed asking if you want to align the re-imported sequence. If you click on **OK**, the sequence is automatically aligned using the **Align to Previous** function (see Section 6.2.2).

6.7.4 Finding Ambiguities or Special Sequences

The **Edit:Find** command in Sequence Alignment allows you to search for specific objects in the alignment after an alignment has been performed.

1. Click on the **Alignment Item** window to make it active.

2. Select **Edit:Find** or <Ctrl + F>. The **Sequence Alignment, Find** dialogue is displayed.

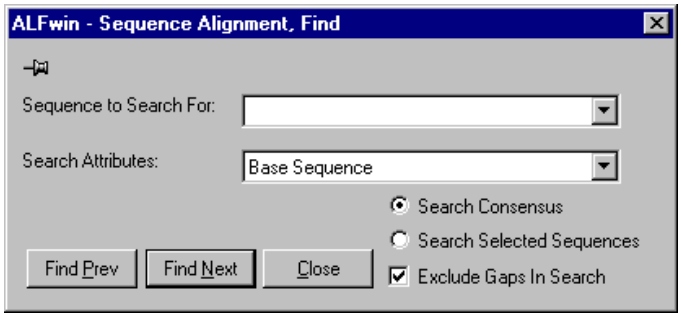


Figure 6-10. Sequence Alignment, Find dialogue.

3. Select the appropriate search object from the **Search Attributes** list:

Base Sequence This allows you to search for a specific nucleotide sequence. The sequence should be entered into the **Sequence to Search For** field. Gaps in the sequence can be included or ignored in the search.

Ambiguity This allows you to locate the next/previous ambiguity.

Mismatches This allows you to locate the next/previous mismatch.

4. Select to conduct the search in either the consensus sequence or the sequences that you have selected in the **Alignment Item** window.

5. Locate the objects using the **Next** and **Previous** buttons as required.

6.7.5 Hiding identical bases

Sometimes it is useful to hide from view those bases which display perfect alignment. ALFwin allows you to replace these positions with spaces or another character so that attention can be focused on the deviations.

To replace identical bases with a non specific character:

1. Select **View:Properties** to open the **ALFwin - Alignment Properties** dialogue.

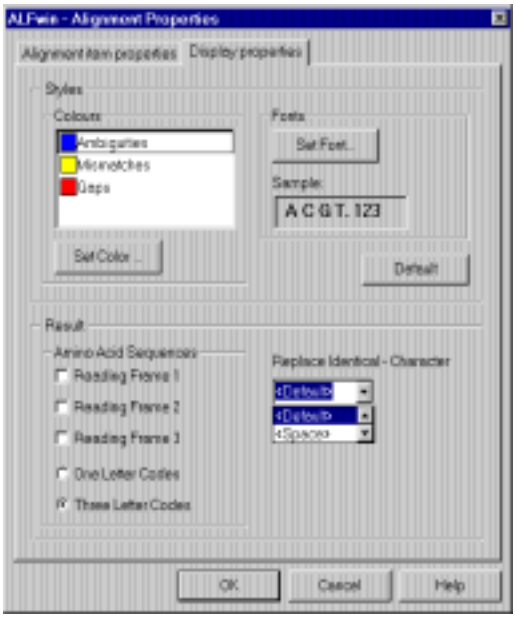


Figure 6-11. Alignment Properties dialogue, Display Properties tab.

2. Select the **Display Properties** tab.

3. Click on the **Replace Identical - Character** list and select the character that you want to use to replace the perfectly matched bases.

Alternatively, type another character that you want here.

4. Select **OK** to close the dialogue and make the changes in the alignment table.

To return the normal display of bases, repeat the above procedure but select **Default** from the options available in the **Replace Identical - Character** drop-down list. When you close ALFwin, the setting is retained and becomes the default when the application is run again.

6.7.6 Disabling the editing functions

The sequence alignment module is supplied with the editing functions enabled. To disable or re-enable the base editing functions, select **View:User Preferences** and deselect or select **Allow Sequence Editing**.

When you close ALFwin, the setting is retained and becomes the default when the application is run again.

6.7.7 Adjusting the number of the first alignment position

By default, the first position in the alignment table is labelled as number 1. You can adjust this start label to allow for bases earlier in the sequences which are not part of the alignment.

To change the starting base number:

1. Select **View:Properties** to open the **ALFwin - Alignment Properties** dialogue.

2. Select the **Alignment Item Properties** tab.

2. Type in the **Start - Label For Bases** field the number of the first base.

3. Select **OK** to close the dialogue and make the changes in the alignment table.

Figure 6-12. Alignment Item Properties tab of the Alignment Properties dialogue.

When you close ALFwin, the setting is retained and becomes the default setting for the next session. When saving an alignment item, the start label is also saved.

6.8 Amino acid sequences

Amino acid sequences can be generated and displayed below the sample sequences in the alignment table. These can be generated for any or all of the three reading frames.

Note: Amino acid sequences can not be edited.

6.8.1 Generating amino acid sequences

The following procedure is used to configure the amino acid display. These settings will hold until the application is closed. If you want to alter the default (startup) configuration, see Section 6.15.2.

To configure the display of amino acid sequences:

1. Select the sequence(s) for which amino acid sequences will be generated. You can select them by clicking their names in the **Label**

list or in the alignment table. Use the <Ctrl> and <Shift> keys with the mouse button to select separate and grouped sequences respectively.

2. Select **View:Amino Acid Sequences** and select (check) one of the three reading frames. Repeat this process to generate amino acid sequences for additional reading frames.

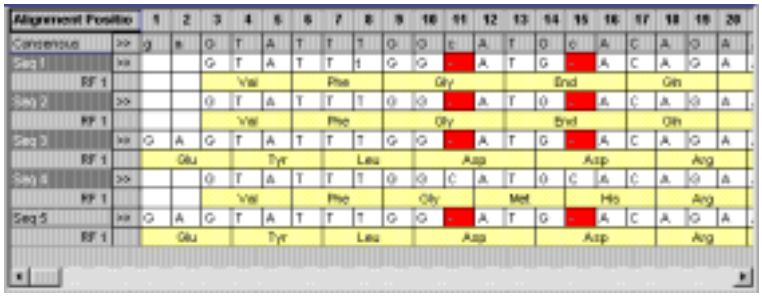


Figure 6-13. Reading frame 1, three-letter code amino acid sequences displayed for the sequences in the alignment table of the alignment item.

3. By default, the amino acid sequences are displayed using the three letter codes. If you wish to change the code, select **View:Amino Acid Codes** and select the **Three-Letter Code** or the **One-Letter Code** option as appropriate.

6.8.2 Recalculating amino acid sequences

When you make changes to the base sequences, amino acid sequences which you have included in your display may need to be adjusted. By default, the necessary recalculations are performed automatically.

To disable the automatic recalculation of amino acid sequences	Select **View:User Preferences** and click on **Automatic Recalculation of Amino Acids** to deselect it.
To initiate a recalculation manually	When automatic recalculation of amino acid sequences is disabled, recalculate by selecting **View:Amino Acid Sequences:Recalculate Amino Seq**.
To restore automatic recalculation	Select **View:User Preferences** and click on **Automatic Recalculation of Amino Acids** to select it.

6.8.3 Changing the translation table

Four different translation tables are provided and can be selected in the **Alignment Item Properties** tab of the **Alignment Properties** dialogue.:

- Standard
- Human Mitochondria
- Neurospora Mitochondria
- Yeast Mitochondria

6.9 The alignment log

The alignment log is initiated when you operate the **New Alignment Item** command from ALFwin Sequence Analyser. The log records:

- the date and time of each logged action
- the User name
- the location of the original file of each sequence included in the alignment
- the stages performed in producing the alignment
- the statistics of the alignment
- editing actions performed on the sequences and bases
- alignment algorithm data.

The log is saved in the .sqa sequence alignment file on completion of the alignment procedures.

To view the log:

1. From the **Sequence Alignment** dialogue select **View:Alignment Log/Notes**. The **Log/Notes** dialogue opens.

2. Select the **Log** tab to view the logged information.

3. Use the close button to remove the dialogue or select another window.

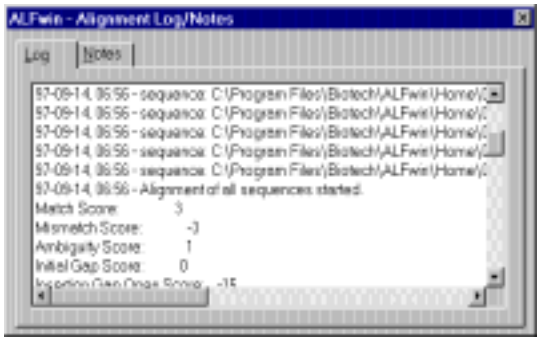


Figure 6-14. Alignment Log/Notes dialogue, Log tab.

6.10 The alignment notes

You use the notes facility to record your own observations while using the sequence alignment module. When you save the alignment file, the notes you make are saved with the rest of the data.

To make notes:

1. From the **Sequence Alignment** dialogue select **View:Alignment Log/ Notes**. The **Log/Notes** dialogue opens.

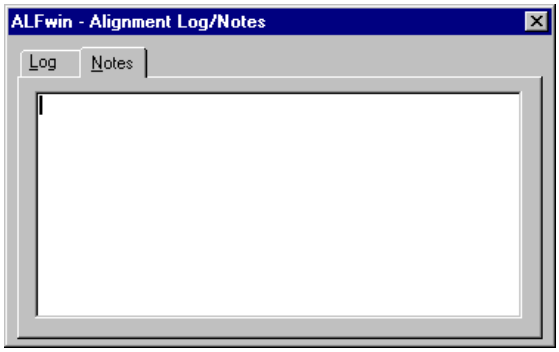


Figure 6-15. Alignment Log/Notes dialogue, Notes tab.

2. Select the **Notes** tab.

3. Type your notes into the **Notes** field.

4. To print the notes, copy them by pressing the <Ctrl + C> keys then paste them into a text application. Use the text application to print the notes.

5. Use the close button to remove the dialogue or select another window.

6.11 Saving the sequence alignment file

A sequence alignment result can be saved on file. The file contains all of the alignment data, the alignment log and notes. The file is recognised as an alignment file by the extension .sqa.

To save the alignment file in the same location using the existing file name, activate the **Sequence Alignment** window and select **File:Save** or click on the **Save** button.

To save using a different name or location:

1. Select **File:Save As**. The **Save As** dialogue opens.

2. Use the dialogue to select the folder where the file is to be saved.

3. Enter a name for the file.

4. Click on **Save**.

6.12 Printing an alignment report

An alignment report is a printout that includes items about selected sequences present in the current alignment item. You select the items you want to include by means of the **Alignment Report Setup** dialogue.

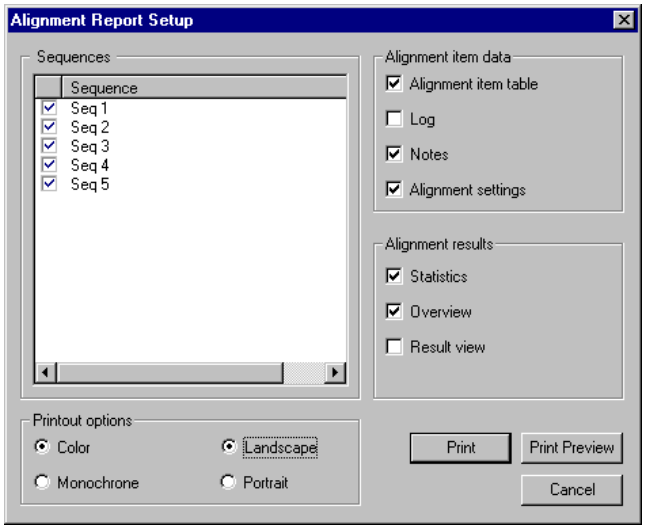


Figure 6-16. Alignment Report Setup dialogue.

To configure and print an alignment report:

1. Select the sequence alignment window to make it active and then select **File:Alignment Report** or click on the **Report** button. The **Alignment Report Setup** dialogue is displayed.

2. Select the **Sequences** to include in the report. Deselect (uncheck) unwanted sequences.

3. Select (check) the **Alignment item data** to be included:

   Alignment item table This includes the list of processed clones present in the alignment list.

   Log This includes information about the alignment automatically generated sequence alignment log.

   Notes This includes information about the alignment notes you have made.

   Alignment settings This includes information about the alignment settings you selected.

4. Select (check) the **Alignment results** to be included:

   Statistics This includes the alignment statistics.

   Overview This includes the graphical information in the alignment overview.

   Result view This includes the alignment table data as displayed on the screen for the whole sequence.

5. Select the **Printout options**:

   Colour or **Monochrome** The default is colour (See Section 6.15.5). Monochrome printouts are useful if you are planning to fax or photocopy the print. Monochrome prints use different shades of grey for the alignment imperfections.

   Landscape or **Portrait** The default is portrait.

6. If you want to close the **Alignment Report Setup** dialogue without printing a report, click on **Cancel**.

7. To view the appearance of the report before printing, click on **Print Preview**.

8. Click on **Print** to send the report to the printer.

The next time the same user opens the **Alignment Report Setup** dialogue, the settings will be the same as in the last used setup.

6.13 Exporting sequence alignment data

You can export alignment sequences to files so that they can be loaded into other applications that allow further evaluation of the data. The formats available depend on the type of data being exported.

Consensus and amino acid sequences can be exported in ASCII format which is accepted by many standard spreadsheet and word processing applications. In MSF or PHYLIP format (see page 6-3) you can export all or only the selected base sequences.

To export alignment sequence(s):

1. In the **Sequence alignment** window, select the base or amino acid sequences to be exported. If you want to export all the sequences, leave the sequences unselected.

2. Select **File:Export Alignment**. The **ALFwin - Sequence Alignment, Export** dialogue is shown.

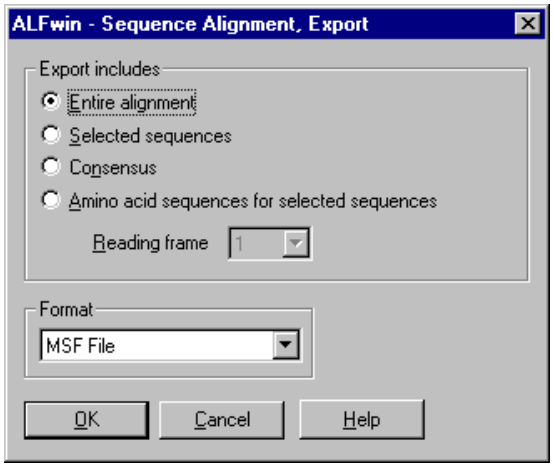


Figure 6-17. Sequence Alignment, Export dialogue.

3. Select from the **Export includes** options the items to be exported:

Entire alignment	To export all the alignment data
Selected sequences	To export the base sequences selected in step 1
Consensus	To export only the consensus sequence
Amino acid sequences...	To save the amino acid sequences selected in step 1. If you select this option, open the **Reading frame** drop-down list and select the desired reading frame.

4. Open the **Format** drop-down list and select the appropriate format from the options available.

5. Click on **OK**. The **Save As** dialogue opens.

6. Use the dialogue to select the folder where the file is to be saved.

7. Enter a name for the file.

8. Click on **Save**.

6.14 Opening a previously saved sequence alignment item

To open a sequence alignment item that you have previously saved:

1. Select **File:Open** or click on the **Open** button. The **Open** dialogue is displayed.

2. Select from the **Files of type** drop-down list, **alignment files (*.sqa)**.

3. Open the folder containing your sequence alignment files. Select the appropriate file and click on **Open**. The sequence alignment window is opened.

Note: If another sequence alignment item was already open, you are allowed to confirm a save of the item before it is closed.

6.15 Configuring the sequence alignment module

The configuration of the sequence alignment module can be adjusted to your requirements. Temporary configuration using **View:Consensus** (see Section 6.5) and **View:Amino Acid Sequences** (see Section 6.8) has already been described. When ALFwin Sequence Analyser is closed the current settings become the default settings for the next session.

The following operations use the **View:Properties** options to change the default configurations.

6.15.1 Configuring the consensus mode

The two consensus modes are described in Section 6.5.1.

To change the default consensus mode:

1. Select **View:Properties** to open the **ALFwin - Alignment Properties** dialogue and select the **Alignment Item Properties** tab.

2. Use the **Consensus Mode** buttons to select **Least Common Denominator** or **Majority**.

6.15.2 Configuring the amino acid display

The amino acid display options are described in Section 6.8.

To change the default display of amino acid sequences:

1. From the **ALFwin - Sequence Alignment** dialogue, select **View:Properties**. The **ALFwin - Alignment Properties** dialogue is shown.

2. Select the **Display Properties** tab.

.

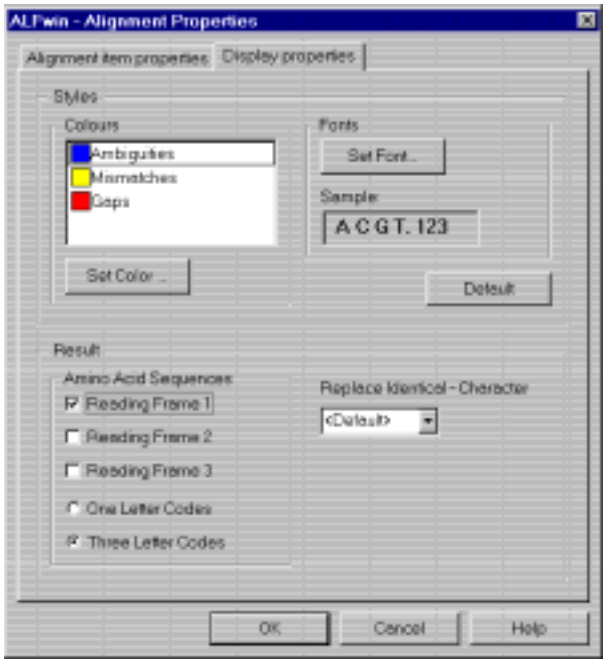


Figure 6-18. Alignment Properties dialogue, Display Properties tab.

3. Use the dialogue to select the reading frames required.

4. Select **One-Letter Codes** or **Three-Letter Codes**.

5. Open the **Interpretation Table** list in the **Alignment item properties** tab and select the table required.

6. Click on **OK** to close the dialogue.

The format selected is applied to all the sequences.You can edit the display of amino acids sequences on one or more of the base sequences but only one of the letter codes can be used throughout (see Section 6.8.1).

6.15.3 Configuring the operation of the alignment algorithm

You can adjust:

- the direction of the sequence data which may be considered for alignment
- the algorithm used for alignment.

To configure the alignment algorithm:

1. Select **View:Properties** to open the **ALFwin - Alignment Properties** dialogue.

2. Select the **Alignment Item Properties** tab.

3. Use the buttons in **Algorithm Options** to select the sequence orientation permitted during alignment.

   Check Both Directions — This is the default setting. When the alignment calculation is performed, the sequences are checked for best alignment in both the forward and complement-reverse directions.

   Check Current Direction — When the alignment calculation is performed, the sequences are checked for best alignment in the direction in which the sequences were imported.

4. Click on the **Algorithm** button. The **ALFwin - Sequence Alignment Algorithms** dialogue opens. Available types of algorithms can be set here:

   The general sequence alignment algorithm considers only two sequences at a time, which means that all the multiple alignment operations are performed in pairs. The included sequences can thus be paired in different ways and, depending on what sequences that are aligned during the calculation, the final alignment may become different. There are a few different theories about how to

combine sequences in the alignment procedure, and they are designed to work with different amounts and qualities of the sequences. Some of the methods suggested work quickly but produce reduced quality of the final alignment. Other algorithms use iterative procedures that take more time, but generate better final alignments. For more details concerning multiple alignment algorithms, consult the paper "Comprehensive study of iterative algorithms of multiple sequence alignment" by Makoto Hirosawa, CABIOS Vol.11 no 1 1995, where comparisons are made between the effect of different multiple alignment algorithms.

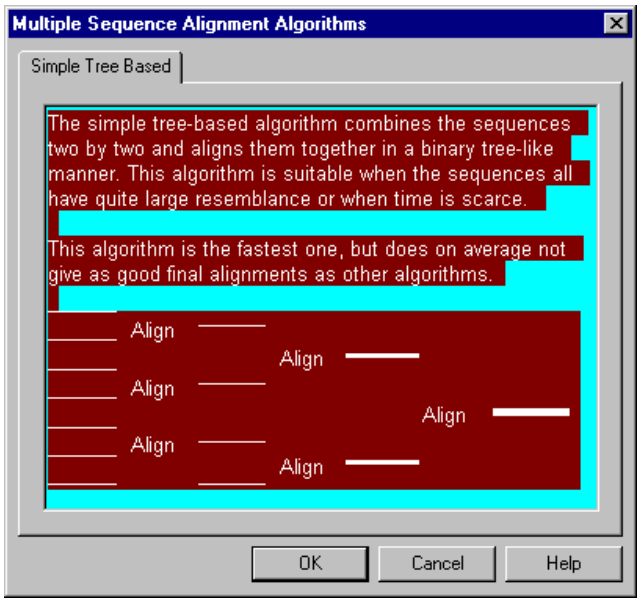

Multiple Sequence Alignment Algorithms
Simple Tree Based
The simple tree-based algorithm combines the sequences two by two and aligns them together in a binary tree-like manner. This algorithm is suitable when the sequences all have quite large resemblance or when time is scarce.
This algorithm is the fastest one, but does on average not give as good final alignments as other algorithms.
Align
Align
Align
Align
Align
Align
OK
Cancel
Help

Figure 6-19. Multiple Sequence Alignment Algorithms dialogue.

In ALFwin 2.00, the **Simple Tree Based** algorithm is applied. This algorithm works quickly and is designed to work best when the quality of the included sequences is about the same. The algorithm is straight forward and teams up the sequences in a binary tree fashion.

5. Click on the **Advanced** button.
   The **ALFwin - Sequence Alignment, Advanced** dialogue opens.

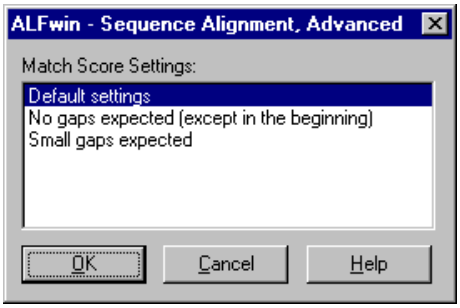


Figure 6-20. Sequence Alignment, Advanced dialogue.

Use this dialogue to select the algorithm settings used by the alignment algorithm. You can select the algorithm according to the number of gaps you expect in the sequences:

- **Default settings**
- **No gaps expected (except in the beginning)**
- **Small gaps expected**

The method used to align any two sequences is suggested by Needleman and Wunsch, 1970, Mol. Biol. 48, 444. It is based on dynamic programming and involves setting quite a few different parameters. The parameters of the algorithm affect the final alignment and, depending on the quality of the sequences to align, different settings of the alignment algorithm parameters should be used.

6. Click on **OK** to close this dialogue.

6.15.4 Configuring data import

Data can be imported in the following formats:

- Amersham Biosciences *.alf or *.alx result files
- STADEN SCF files (only the sequences are imported)
- ASCII files.

ALFwin data files include information about the statistical significance of bases they contain and you can configure the import procedure to import all the bases or just the upright bases.

SCF and ASCII files do not include information about the quality of bases. When you import these files, all bases are always imported and the import configuration has no effect. The statistical significance of the bases included in these files can thus not be guaranteed.

To configure the import scheme for ALFwin data files:

1. Select **View:Properties** to open the **ALFwin - Alignment Properties** dialogue.

2. Select the **Alignment Item Properties** tab.

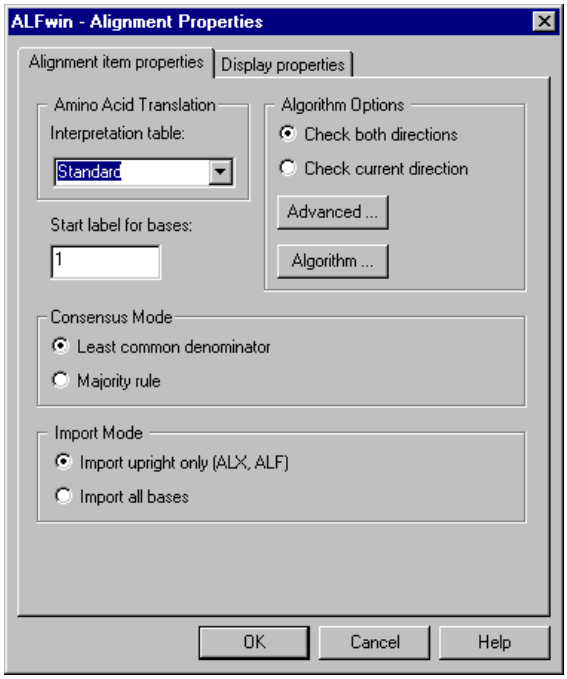


Figure 6-21. Alignment Item Properties tab of the Alignment Properties dialogue.

3. To import only upright bases from *.alf and *.alx files, select **Import Upright Only**. Otherwise select **Import All Bases**.

6.15.5 Changing the colour settings

Ambiguities, mismatches and gaps in the alignment table and the alignment overview are highlighted and identified by the use of colour.

To alter the colours:

1. Select **View:Properties** to open the **ALFwin - Alignment Properties** dialogue.

2. Select the **Display Properties** tab.

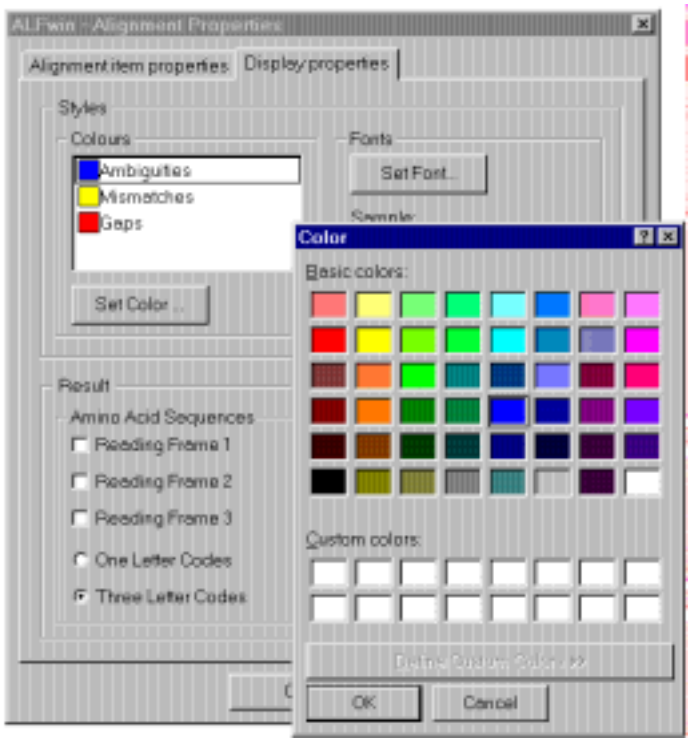


Figure 6-22. Alignment Properties dialogue, Display Properties tab and open Colour dialogue.

2. Select the appropriate item under the **Colours** list and click on the **Set Colour** button. The **Colour** dialogue is shown.

3. Select a colour.

Note: You should select different colours so that it is easy to distinguish different discrepancies. Also select colours that allow the inverted colours used for the text to be clearly displayed.

4. Click on **OK** to close the **Colour** dialogue.

5 Click on **OK** to change the colour.

6.15.6 Changing the font settings

To configure the font used in the alignment table:

1. Select **View:Properties** to open the **ALFwin - Alignment Properties** dialogue and select the **Display Properties** tab. Click on the **Set Font** button. The **Font** dialogue is shown.

2. Select a **Font**, **Font Style** and **Size**.

3. Click on **OK** to close the **Font** dialogue.

4. Click on **OK** to change the font.

If you want to restore the default colour and font settings, click on the **Default** button.

7 Troubleshooting

This chapter helps you identify and correct operational problems with ALFwin. In all cases, a reason for the encountered problem is suggested and, if appropriate, a course of action is proposed.

General hints using ALFwin Software

Menu command cannot be found.

Problem: Menu command inactive (greyed out)

Explanation: The wrong window or part of the window is activated.

Solution: Click the part of the window you want to work with by clicking on it.

The computer is working slowly & Error messages concerning low memory resources

Problem: The computer is working slowly. Error messages like "Out of memory..., "Low memory resources.." etc. may appear.

Explanation: Not sufficient RAM memory or disk space left. You may be running to many applications at the same time.

Solution: Try to close down programs / files you are not using. If you can not do this, then shut down and restart the computer. If your computer has a small hard disk (<300 MB) perform the shut down and restart operation on a regular basis or between runs.

Error messages concerning file handling, corrupt (program) files

Problem: Error messages like "Required file xx.xxx was not found" or "Error occurred while initialising the algorithm settings" appears.

Solution: Re-install the ALFwin Software.

Printing problems

Problem: Last curve window on each page not properly printed.

Explanation: Insufficient memory installed in printer.

Solution: Increase the memory of the printer or use a recommended printer (see Section A.2). For more information on printing problems see the appropriate web site for the computer dealer, for example *www.hp.com*.

ALFwin Instrument Control

Connection problem

Problem: "Looking for instrument, please wait..." does not disappear after a few minutes of starting ALFwin Control.

Explanation: There is probably a problem linking to an instrument on the designated COM port.

Solution: Click on **Cancel** in the message window. A dialogue asks if you want to stop waiting. Click on **No**. Check the connections between the instrument and the computer. If the connection is fine, check the instrument setup in the ALFwin Administrator and change COM port setting. Start ALFwin Control again. If you click on **Yes**, you can only edit a casebook but not start a run.

Comment: If you answer **No** to the question “Stop waiting?”, the dialogue “Looking for instrument...” is displayed again. To be able to quit you must answer **Yes**.

Set temperature in casebook is not reached.

Problem: Instrument temperature is 40°C, but the set temperature in the casebook is different.

Explanation: The experimental conditions in the casebook are not activated.

Solution: Click on **Preset** and the temperature setting is sent to the instrument. If not sufficient, see instrument manual.

Flashing light on the Control icon

Problem: The coloured dot on the (minimized) Control icon is flashing.

Explanation: Run is paused due to some problem indicated by an error message.

Solution: Maximize the window and view the displayed error message. Take care of the problem and press **Continue**.

Exclusion of lanes have no effect

Problem: Excluded lanes in the **Sample Information** tab in the casebook do not seem to have any effect in the **Curve** window.

Explanation: You have closed the casebook and opened it again. (The excluded lanes information is not saved when the casebook is closed).

or

You have probably ended the run, opened the casebook again and restarted the run. (The excluded lanes information is not saved between runs.)

Solution: End the run, open the casebook again, re-enter the excluded lanes information and restart the run.

Run Conditions set values are not reached

Problem: Run conditions such as **Voltage**, **Current** and/or **Power** in the **Manual** window do not reach the set values.

Explanation: Due to the chemistry of the electrophoretic separation, only one of the parameters will set the limit of the system. This one will reach (or almost reach) the set value, but the other two will not.

Solution: Check only the parameter that limits the system.

ALFwin can not find the new run

Problem: After the completion of a run, the ALFwin Sequence Analyser module can not find the file for that newly completed run.

Explanation: Possibly due to too heavy loading on the computer during the end of the run.

Solution: Start the same ALFwin Control module as the run was made in and log in as same user as before. After this "treatment" the file will be possible to open from ALFwin Sequence Analyser.

ALFwin stops collecting data during a run

Problem: ALFwin stops collecting data during run.

Explanation: A local (language) version of Windows 95 is used or resource conflict in the computer.

Solution: We recommend using the International (UK) version of Windows 95 together with ALFwin since the UK version is the only one that ALFwin has been developed and tested against. See also recommended system requirements, section A.1.

Error message occurs during an ALFwin run

Problem: During run a "Control" error message occurs such as "This program has performed an illegal operation and will shut down. If the problem persists, contact the program vendor. CONTROL caused an invalid page fault in module MFC42.DLL"

Explanation: The operation system may sometimes interfere with ALFwin Control (or vice versa).

Solution: During run, start up the Control module again and log on as the same user. If the instrument's green LED has not started to flash, the data collection will continue. Please note that on the screen it will look as if all data prior to the failure is deleted. However, all data prior to the error message will be added to the data collected after the failure, when the run is ended. That means that a complete file will be possible to open in ALFwin Analyser after the run.

ALFwin Sequence Analyser

No processing result

Problem: Peaks are seen but the algorithm failed to start.

Explanation: The algorithm usually needs a clear primer peak to be able to start its operation. This problem may appear in solid phase sequencing, since it shows just a minor primer peak.

Too extensive sampling, i.e. too many data points per peak, may interfere with "downstream" processing since the processing is optimised for a certain number of data points per peak.

Solution: Start processing manually.

Use correct Sampling interval, i.e. sampling every 2 sec for the standard cassette and 0.5-1 sec for shorter gels that run very fast.

The base calling algorithm has stopped

Problem: You have got shorter reading length than expected.

Explanation: Many different reasons are possible when the base calling algorithm has stopped early. "Smiling effects", chemistry problem, template quality etc.

Solution: Use manual start time. Apply shift (**Edit: Apply Shift**), perform shift and process again. Check further reasons in the main trouble shooting guide for ALF*express*.

The base calling seems to be totally wrong

Problem: You realise that the evaluation is totally wrong.

Explanation: The selected lane order is wrong, you have forgotten to exclude unused lanes or excluded lanes with samples.

Solution: None. Perform the run again.

Strange fonts when you use copy clone or export to metafile

Problem: Strange fonts when you use copy clone or export to metafile format.

Explanation: The export uses an export layout that depends on height and width.

Solution: Copy or export with maximized window.

Corruption of data during transfer from one drive to a network drive

Problem: After editing the result file, it was saved to a network drive. Next time the file was opened it was found to be corrupt i.e. not possible to open.

Explanation: This problem can occur if the disk space is limited on the drive where the file was attempted to be stored. It is not possible to secure disk space on the network drive, due to the fact that a lot of different network users may use up space at the same time.

Solution: It is not possible to restore a file that has been destroyed in this way. To avoid the problem, always save the file to a disk with sufficient space. Copy the file to the network drive. Check that the copied file was correctly saved, then delete the file on the computer hard disk.

Error message: Too many samples in lane

Explanation: When trying to create an ALF or SCF file with too much data from the source file.

Solution: Use the alx format for larger data quantities.

Sequence Alignment

Error message: Command failed.

Explanation: No clear explanation is available.

Solution: Restart ALFwin Sequence Analyser before applying the command again.

Error message: An error occurred in the pair wise alignment algorithm, check that the algorithm settings are OK

Explanation: An erratic algorithm setting may have been supplied to the alignment algorithm, or that not enough memory exists for the alignment to be performed.

Solution: Try to set another algorithm setting or to release memory by closing other applications or closing files in Sequence Analyser.

A System requirements

A.1 Computer

Computer	Compaq PC, Pentium/233 MHz (minimum Pentium/75 MHz for one instrument)
RAM memory	64 MB (minimum 32 MB)
Recommended free hard disk space	300 MB if running two ALF*express* systems simultaneously
Operating system	Windows 95, international edition, including Service release 2 (4.00.950b).
Graphics	800 x 600 pixels with 64k colours (minimum 800 x 600 pixels SuperVGA graphics adapter, 256 colours)
Serial ports	2 serial ports are recommended (allows two instruments to be run).

A.2 Supported printers

The following printers have been tested for compatibility with ALFwin Sequence Analyser 2.00:

HP LaserJet 4M and 5MP.

HP DeskJet 870Cxi and 890CXi.

B Short guide to Windows 95

This appendix gives a brief introduction to Windows 95 for beginners. For further information, you are recommended to read the Microsoft publications for using Windows 95 and other associated literature.

The following topics are covered:

- Starting Windows 95 and logging on
- What is on your screen
- Getting started in Windows 95
- If you have used an earlier version of Windows

B.1 Starting Windows 95 and logging on

1. Turn on the computer and Windows 95 will be automatically started.

2. If your computer is connected to the network, you will be prompted to enter your user name and password. Enter these and click on **OK**.

   Alternatively, if you do not want to be connected to the network click on the **Cancel** button. You will now only be able to work locally using the computer's hard disk.

3. You may also be asked to supply your user name and password for Windows 95 if you have supplied these before.

   However, if you are using the computer for the first time, you must enter a user name and password in the **User name** dialogue. If you do not want enter the password each time you start the computer leave the **Password** field blank and click on **OK**. When prompted to confirm the password, leave all fields blank and click on **OK** again.

B.2 What is on your screen

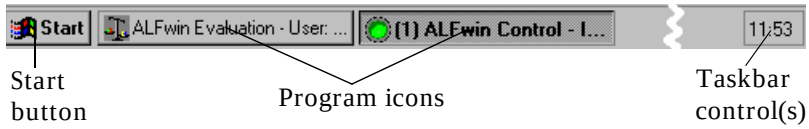
Depending on how your computer is set up, various items appear on your screen when you start Windows 95. Here are a few of the important items:

My Computer Double-click this icon to view the hardware and software content of your computer.

Network Neighbourhood If your computer is connected to the network, double-click this icon to see available network resources.

Start button You can click the Start button in the lower left corner on the Windows 95 taskbar to launch a software application, open a document, change the system settings, get help, find items on your computer, and more (see Section B.3.1).

Windows 95 taskbar The Start button is located on the taskbar for launching programs. The taskbar also shows an icon for each of the applications that you open. To switch between applications, click on the icon for the application you want. When you close a window, its icon disappears from the taskbar.



Recycle Bin The Recycle Bin is a temporary storage place for deleted files. You can use it to retrieve files deleted by mistake.

B.3 Getting started in Windows 95

This section gives you a quick overview of getting started in Windows 95.

B.3.1 Start button

The Start button is in the lower left corner of your screen located on the Windows 95 taskbar. When you click on the Start button, a menu cascade is displayed containing everything you need to begin using Windows 95.

Programs	Displays a list of programs you can start.
Documents	Displays a list of documents that you have opened previously.
Settings	Displays a list of icons that can be opened to allow you to change the settings for your computer.
Find	Enables you to find a file, folder, file shared computer or mail message. Here you can search, for example, the .alx files for different runs.
Help	Starts Windows 95 Help. You can then use the Help Contents, Index, or other tabs to find out how to perform a task in Windows 95.
Run	Starts a program or opens a folder when you type an MS-DOS command.
Shut Down	Shuts down or restarts your computer, or logs you off. If you have installed and later closed other applications, it can be useful to shut down and restart the computer to clear memory.

B.3.2 Basic mouse functions in Windows 95

Use the mouse to select items, activate items, drag and drop items or view right mouse button command options:

Selecting items	Place the mouse pointer over the item of interest and click once with the left mouse button to select it - note the grey shading of the selected item. To select several items on the desktop, either hold down the <Ctrl> key on the keyboard when you select items, as described above. Alternatively, place the mouse pointer anywhere outside of the icons of interest and click and hold the left mouse button. Move the mouse pointer to drag a

	box around the icons of interest and release the mouse button. All of the items are selected.
Activating items	To view the contents of a folder on the desktop, double click on it with the left mouse button. Similarly, to launch a software application, double click on its icon.
Dragging and dropping items	You can move icons around by placing the mouse pointer on an icon, pressing down and holding the left mouse button, moving the mouse button together with the icon to a new place and releasing the mouse button to drop the icon. This is useful for moving files between folders and to or from the Recycle Bin.
Right mouse button menus	Investigate various command options for an icon and an active window by clicking on it with the right mouse button. Make the appropriate selection from the menu that is displayed. These command options are the same as those offered in the menu **View:Properties** options in ALFwin.

B.3.3 Using a dialogue box

Windows 95 displays a dialogue box when you must supply information or specify options or properties. There are different types of options:

Radio buttons	Circular buttons allowing you to select one option only at a time.
Check option boxes	Square boxes that you can click in to place a check mark for the one more options you want.

Text fields	Empty boxes allowing you to insert either free text or parameters within a defined range.
Option buttons	Buttons allowing you to execute or retract the implementation of the selected options within a dialogue.

B.3.4 Sizing windows

You can adjust the size of a window to accommodate viewing several windows at the same time.

There are two methods you can use to size windows:

- Use the buttons in the upper right corner of the window to Minimise (view the taskbar icon only) or Maximise/Return to previous size.
- Drag the border of the window by pointing at the window border, pressing and holding the left mouse button and dragging the window border to appropriate size.

B.3.5 Closing a window

To close a window or exit a program, click the close button (button with a cross) in the upper-right corner of the window, next to the Minimise and Maximise buttons.

B.3.6 Moving windows

You can move windows on the screen by pointing at the window title bar, pressing and holding the left mouse button and dragging the window to its new position.

B.3.7 Using toolbars

Toolbars (bars with buttons) appear in many application software and provide quick ways to perform tasks. Most toolbar buttons correspond to available menu commands.

B.3.8 Scrolling windows

If a window is not large enough to display all the information, a scroll bar appears at the side and/or bottom of the window. You can drag the scroll box or click on the scroll arrows.

B.3.9 On-line Help

Use the on-line help function if you need help. You can find help for Windows 95 under the Start button. Most software applications also have extensive help facilities, accessed either from the **Help** menu in the program or by clicking on a **Help** button in a dialogue.

B.3.10 Creating a shortcut

You can create a shortcut to a program thus enabling you to click on an icon on the Windows 95 desktop to start the program.

1. Place the mouse pointer anywhere on the Windows 95 desktop, but not on an icon, and click on the right mouse button. A pop-up menu is displayed.

2. Select **New** and then **Shortcut**. The **Create Shortcut** dialogue is displayed.

3. Click on the **Browse** button to display the **Browse** dialogue. Locate and select the program file for which you want to create a shortcut. Click on **Open** to return to the **Create Shortcut** dialogue.

4. Click on the **Next** button to display the **Select a Title for the Program** dialogue. Enter a name for the program if different to the one displayed.

5. Click on **Finish** and the dialogue closes. A shortcut icon to the program will appear on the desktop.

6. To start the program using the shortcut, double click on the icon.

B.4 If you have used an earlier version of Windows

For those of you who have used an earlier version of Windows, typically Windows version 3.1, a few of the common functions have been changed.

Windows 3.x.x function	Windows 95 equivalent
Program Manager	Start button on the Windows 95 taskbar.
File Manager	Windows Explorer; located in the Start button menu under Programs.

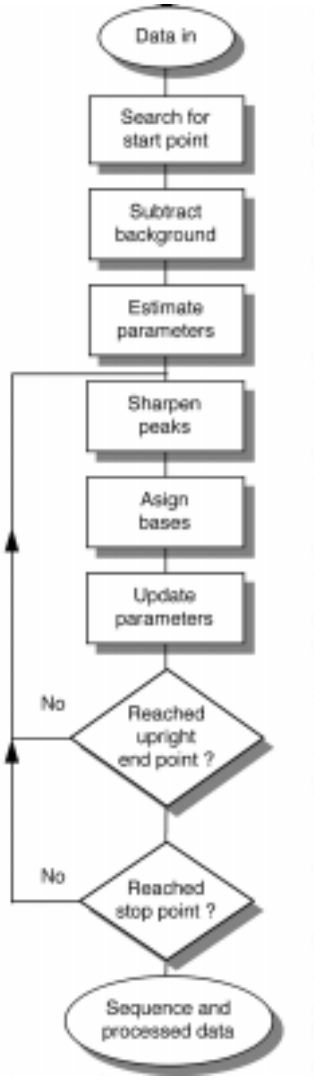
MS-DOS Prompt	MS-DOS Prompt; located in the Start button menu under Programs.
Control Panel	Control Panel; located in the Start button menu under Settings.
Print Manager	Printers; located in the Start button menu under Settings.
Run Command	Run; located in the Start button menu.

C The base calling algorithm

This appendix describes the base calling algorithm used to process the raw data from a sequencing run.

C.1 How the base calling algorithm works

The algorithm is described in the following flow chart.



Each position in the flow chart represents the following:

Search for start point	The algorithm searches automatically for a suitable start point. A start and a stop point can also be defined manually.
Background subtraction	The raw data signal contains a slowly varying offset value often referred to as the background. At this stage, the background component is calculated and subtracted from each lane to simplify further processing.
Estimate parameters	The algorithm makes an initial estimation of the parameters (e.g. time-shift, peak shape and peak distance). This is done by analysing the region after the start point.
Sharpen peaks	The signal is filtered through a digital filter that enhances the resolution of the signal. This separates individual peaks into peak clusters (groups of peaks).
Assign bases	The bases within a limited region are assumed to be fairly equally spaced. The algorithm is able to estimate where the next base will appear. The symbol of the base in each position is chosen according to the following rules: If there is a peak in a certain lane near the assumed position and no peaks in the other lanes, the symbol corresponding to the lane is chosen. If instead, a significant peak is found near the position in more than one channel, an ambiguity symbol is chosen. If there are peaks in all four channels near the position, it is considered a full stop and the symbol “N” is chosen.

If there are no significant peaks near the position or if neighbouring peaks are too close, the existence of a base is questioned and a lower-case version of the symbol is chosen. This is called a frame ambiguity. A peak is considered to be near a position if its height at the position is high enough or if it has its maximum close enough to the position.

Update parameters

After each base has been assigned, the parameters are re-tuned so that they are adapted to local changes in the signals.

Upright end point

The algorithm estimates the point in the sequence after which the error frequency might become bigger than 1%. This point is called the upright end point. At this point, at least one of the following criteria is satisfied:

The peaks in the raw data are too poorly resolved.

There are too many ambiguities in a subsequence of a certain interval.

There are too many ambiguities in a limited interval after a frame ambiguity.

The peaks are not well resolved and there is a long interval without any well-shaped and resolved peaks.

Stop point

After the upright end point has been reached, another 300 bases are read to a point called the stop point. The bases that are read after the upright end point might have an error frequency higher than 1% and are therefore written in italics.

C.2 How to use the base calling algorithm

The base-calling function is constructed to work with minimum interaction; you can normally generate high quality data by using the standard settings. In some cases, however, the quality of the data can be improved by overriding the default settings for the base-calling process. This is done by providing the base-calling program with additional information. The most important examples of user interactions are: (a) setting the start and/or the stop point for processing, (b) manually adjusting the time shifts, and (c) choosing one of the heterozygote modes instead of the normal mode for processing. These three user interactions are described in the following sections.

C.2.1 When to set the start and/or stop point manually

Processing can be set manually either by using the post-run action function in ALFwin Control or the process function in ALFwin Evaluation. Reasons for setting the start point manually include:

- the data of interest is proceeded by another sequence, e.g. by a vector sequence. It may be easier to find the data of interest if the processing starts just before it.
- to make sure that the manually adjusted time shifts will be used correctly. See also Section C.2.2.

Setting the stop point ensures that no processed data or sequence will be generated after that point. This saves disk space when storing the results. Situations when it can be useful to set the stop point manually include:

- when it is known that there is no data of interest after a certain time, for example, when the samples are PCR fragments with a limited maximum length
- when the data are not interpretable after a certain time.

C.2.2 When to manually adjust the time shift before processing

If there are differences in the mobilities of the DNA fragments in different lanes belonging to the same clone, the base-calling program may have problems in automatically compensating for the resulting time shifts between the A,C,G and T-signals. This problem is most common in the outermost clones of the gel. In such cases it is appropriate to manually adjust the shift before processing the clone. It is often a good idea to manually set the wanted start point for the processing to ensure that the base-calling starts in the interval where the shift has been adjusted. To make the base-calling program use the manually adjusted shifts, **Use applied shift** must be selected in the **Process Setup** window before the processing is carried out. You can also select **Use these shifts** and enter the required shifts from the keyboard. This function can be used to reproduce an earlier processing with a certain time shift.

C.2.3 The different types of processing

In addition to the normal processing mode, there are three heterozygote modes for processing; Low, Medium and High. Using one of the heterozygote modes increases the tendency to call ambiguity symbols. This tendency is strongest in the High mode. We recommend you use one of the heterozygote modes when it is important that all ambiguous positions in the raw data are found, such as when looking for heterozygocity in genes in DNA samples from diploid organisms. The possible heterozygote positions are easily traversed by using the Find Next Ambiguity search function. Notes about the positions of interest can be written in **Evaluation Notes** in the casebook.

C.3 Base ambiguity codes

The IUPAC (ambiguity) codes for unresolved base positions are listed below:

Code:	Unresolved bases:
M	AC
R	AG
W	AT
S	CG
Y	CT
K	GT
V	ACG
H	ACT
D	AGT
B	CGT
N	ACGT

D Keyboard shortcuts

D.4 ALFwin Control

D.4.1 General

F1	Help.
Ctrl+N	Create new Casebook.
Ctrl+O	Open an existing casebook. The **Open** dialogue box is shown.
Ctrl+P	Print casebook. The **Print** dialogue is shown.
Alt+F4	Exit ALFwin Control.
Ctrl+Tab or Ctrl+Shift+Tab	Switch between **Curve**, **Manual** and **Logbook** windows.

D.4.2 In the Curve window

Ctrl+1-9 or 0	Clone/group 1-10 is displayed.
Ctrl+Shift+1-9 or 0	Clone/group 11-20 is displayed, if it exists.
Ctrl+Alt+1-9 or 0	Clone/group 21-30 is displayed, if it exists.
Ctrl+Alt+Shift+1-9 or 0	Clone/group 31-40 is displayed, if it exists.
F7	Zoom in by large steps.
F8	Zoom out by large steps.
Shift+F7	Zoom in by small steps.
Shift+F8	Zoom out by small steps.
F9	Reset zoom.
Ctrl+Page Down or Ctrl+End	Move zoomed area to the end of the run.

Ctrl+Page Up or Ctrl+Home	Move zoomed area to the beginning of the run.
Page Up	Move zoomed area one screen to the left.
Page Down	Move zoomed area one screen to the right.
Left or right arrow key	Move zoomed area one tenth of a screen to the left or right.
Ctrl+Shift+S	Show signal levels. The **Signal Level** dialogue is shown (Curve mode).

D.4.3 In the Manual window

Tab or Shift+Tab	Move between experimental conditions and buttons.

D.5 ALFwin Evaluation

D.5.1 General

F1	Help.
Ctrl+O	Open result. The **Open** dialogue box is shown.
Ctrl+S	Save result under the existing name.
Ctrl+P	Print the selected **Gel view** or **Curve** window. The **Print** dialogue box is shown.
Ctrl+Shift+P	Print all open **Curve** windows. The **Print** dialogue box is shown.
Ctrl+F4	Close the active result.
Alt+Tab	Switch between opened programs.
Alt+F4	Exit ALFwin Evaluation.
Alt+underlined letter	Select commands in menus or in dialogue boxes.

Ctrl + N	New alignment file.
Ctrl + R	Start ALFwin Control so that a new run can be made.
Tab	Move forward between fields in the active dialogue box.
Shift+Tab	Move backwards between fields in the active dialogue box.
Ctrl+Tab	Move between open windows.
Home or End	Scroll/move to the beginning or end of view or list.
Page Up or Down	Scroll/move to next page, up or down, in view or list.
Arrow keys	Scroll/move one step in view or list.

D.5.2 In the Clone view (in the Result window)

Up or down arrow key	Move up or down in the clone list.
Right or left arrow key	Show or hide lanes in the selected clone.
Ctrl+Enter	Open the selected clone.

D.5.3 In the Gel view (in the Result window)

F5	Show raw data.
F6	Show processed data.
F7	Zoom time, in.
F8	Zoom time, out.
Shift+F7	Zoom channels, in.
Shift+F8	Zoom channels, out.
F9	Reset zoom.

F10	Activate Zoom with Mouse mode. Press again to de-activate this mode.
Ctrl+C	Copy **Gel view** to clipboard.

D.5.4 In the Curve window

Ctrl+Tab	Switch between open windows.
Ctrl+1-9 or 0	Clone 1-10 is displayed.
Ctrl+Shift+1-9 or 0	Clone 11-20 is displayed, if it exists.
Ctrl+Alt+1-9 or 0	Clone 21-30 is displayed, if it exists.
Ctrl+Alt+Shift+1-9 or 0	Clone 31-40 is displayed, if it exists.
F5	Show raw data.
F6	Show processed data.
F7	Zoom in.
F8	Zoom out.
F9	Reset zoom.
Alt+<	Show complementary and reverse curves.
Ctrl+Shift+A, C, G or T	Hide or show lanes A, C, G or T.
Ctrl+Shift+S	Show signal levels. The **Levels** dialogue is shown.
Ctrl+E	Activate or inactivate Edit mode. The Edit toolbar is shown or hidden. Note that Replace mode is activated first.
Ctrl+Y	Activate or inactivate Apply Shift mode. The Shift toolbar is shown or hidden.
Ctrl+F	Find ambiguity or sequence. The **Find** dialogue box is shown.

Ctrl+C	Copy clone, sequence or gel depending on the active part of the screen.
Ctrl + L	Link open **Curve** windows for similar presentation of curve views.
Ctrl+ Home	Move to the first base in the run. When the sequence is reversed, move to the last base in the run.
Ctrl+ End	Move to the last base in the run. When the sequence is reversed, move to the first base in the run.
Page Up	Move zoomed area one step to the left.
Page Down	Move zoomed area one step to the right.
Left or right arrow key	Move to base one step to the left or right.

D.5.5 In the Curve view (in the Curve window)

Home	Move to the first base in the sequence shown in the curve view. Always counts from the left.
End	Move to the last base in the sequence shown in the curve view. Always counts from the left.

D.5.6 In the Orientation view (in the Curve window)

Curve and bar mode

Home	Move to the beginning of the run. When curves are reversed, move to the end of the run.
End	Move to the end of the run. When curves are reversed, move to the beginning of the run.
Left or right arrow key	Move to base one step to the left or right.

Text mode

Home	Move to the first base in the marked part of the sequence. Always counts from the left.
End	Move to the last base in the marked part of the sequence. Always counts from the left.
Arrow keys	Move to base one step to the left or right, or up or down.
Ctrl+C	Copy the sequence to clipboard.

D.5.7 In the Edit mode (after Ctrl+E in the Curve widow)

Insert	Switch between Replace and Insert mode.
Delete	Delete marked base.
Ctrl+left or right arrow keys	Move the position of selected base to the left or right. Note that the curve view and not the orientation view has to be activated.
Ctrl+Z	Undo last entry or move.

D.5.8 In the Process Status window

Ctrl+Enter	Open the selected clone(s).

D.5.9 In the Casebook

Shortcuts within the casebook:

Tab	Move forward between fields in the current page.
Shift+Tab	Move backwards between fields in the current page.
Ctrl+Tab or Shift+ Ctrl+Tab	Move between pages in the casebook.

Shortcuts within the casebook Sample Information page:

ALT+C	Switch to the clone tab.
ALT+L	Switch to the lane tab.

CTRL+C	Copy information from selected cells.
CTRL+X	Cut information from selected cells.
CTRL+V	Paste information into cells.

An activated cell is a cell where the cursor is visible. A cell that has the black box around it but no selected text or visible cursor is not an activated cell.

The action of keyboard keys depends on the activated or deactivated status of the cell(s).

	With deactivated cells	*With activated cells*
Tab	Move to the next cell.	Confirm the cell contents. If valid, move to the next cell.
Return	Activate the cell, select all text in the cell and position the cursor at the end of the selected text.	Confirm the cell contents. If valid, move to the next cell.
Home	Activate the cell and position the cursor at the beginning of the text.	Position the cursor at the beginning of the text.
End	Activate the cell and position the cursor at the end of the text.	Position the cursor at the end of the text.
Delete	Remove all cell contents and activate the cell	Remove all selected text in the cell. If none is selected, remove the character right of the cursor.
Escape	Nothing	Cancel any cell edits and deactivate the cell.
Left arrow	Activate the cell and select all text. The cursor is positioned at the beginning of the text.	Move one character to the left.

Right arrow	Activate the cell and select all text. The cursor is positioned at the text end	Move one character to the right.
Up arrow	Move to the cell above	Validate the cell. If it is valid, move to the cell above.
Down arrow	Move to the cell below.	Validate the cell. If it is valid, move to the cell below.
Ctrl+ Home	Move to the top left cell.	Validate the cell. If it is valid, move to the top left cell.
Ctrl+End	Move to the bottom right cell.	Validate the cell. If it is valid, move to the bottom right cell.
Page up	Move up one page.	Validate the cell. If it is valid, move up one page.
Page down	Move down one page.	Validate the cell. If it is valid, move down one page.

D.6 Sequence Alignment

Ctrl + C	Copy selected bases.
Ctrl + F	Activate **Find Sequence** dialogue.
Ctrl + I	Toggles Edit mode between Insert and Replace mode.
Ctrl + N	New alignment item.
Ctrl + O	Open file.
Ctrl + Z	Undo last edit operation.
Delete	Deletes a highlighted set of bases or deletes a sequence in the alignment item list.

E Reference list

E.1 References for export formats

Format	*Description*	*Further information*
GCG	The .seq format for GCG, the sequence analysis program from Wisconsin University.	http://www.gcg.com/
STADEN SCF	The .scf format for the Staden sequence analysis package. SCF format includes curve data.	Staden, Rodger 1982. An interactive graphics program for comparing and aligning nucleic acid and amino acid sequences. Nucl. Acids Res. 10(9):2951-2961. http://www.mrc-lmb.cam.ac.uk/pubseq/
PC/GENE	The N prefixed format for PC/GENE, a sequence analysis program by IntelliGenetics from the Oxford Molecular group.	http://www.oxmol.com/
ASCII	ASCII .txt format that will load into many standard commercial applications.	-

EMBL	The .eml format for Bioresearch, the sequence analysis program by Fujitsu Ltd.	Information on the EMBL format can be found at several web sites. One example is : http://www.psc.edu/general/software/packages/seq-intro/emblfile.html
DNAsis	The .dna format of DNAsis, a sequence analysis program from Hitachi Software Genetics Systems Group.	http://www.hitachi-soft.com/gs/
MSF	MSF is the multiple sequence alignment format of the GCG sequence analysis package.	http:www.embl-heidelberg.de/predictprotein/new/Dexa/optin_msfDes.html
PHYLIP	PHYLIP (the PHYLogeny Inference Package) is a package of programs inferring phylogenesis (evolutionary trees).	http://evolution.genetics.washington.edu/phylip.html

E.2 Other references

1. For more details concerning multiple alignment algorithms, please refer to "Comprehensive study of iterative algorithms of multiple sequence alignment" by Makoto Hirosawa, CABIOS Vol.11 no 1 1995, where comparisons are made between the effect of different multiple alignment algorithms

2. The method used to align any two sequences is suggested by Needleman and Wunsch, 1970, Mol. Biol. 48, 444. It is based on dynamic programming and involves setting several different parameters. The parameters of the algorithm affect the final alignment and, depending on the quality of the sequences to align, different settings of the alignment algorithm parameters should be used.

3. ALFwin Sequence Analyser 2.00 is licensed under U.S. Patent No.4,558,302 and foreign counterparts " (License from Unisys to use TIFF-LZW graphics)

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Short instruction - ALFwin Sequence Analyser 2.00

Perform a run

1. Turn on the instrument and computer
2. Place the gel cassette into the instrument
3. Start ALFwin Control

4. Define a sequence analysis casebook
5. Preset the instrument conditions
6. Load samples
7. Realign the laser
8. Start the run

Analyse the result file

1. Start ALFwin Sequence Analyser
2. Open a result file

3. Check the gel picture

4. Check the curve view

5. Process the raw data for selected clones
6. Check the results after processing

If necessary adjust the sequence result by:
Applying shift and process again
Setting manual start time and process again
Editing bases manually

Ambiguity codes

Code:	*Unresolved bases:*
M	AC
R	AG
W	AT
S	CG
Y	CT
K	GT
V	ACG
H	ACT
D	AGT
B	CGT
N	ACGT

Align the sequences

1. Open result files containing sequences and/or import sequences
2. Create a new analysis item

3. Align the sequences

4. If necessary edit the sequence alignment result
5. Generate the amino acid sequence in the desired reading frame

Saving and printing

Save (**File:Save**) (<Ctrl + S>)

Save As (**File:Save As**)

Print (**File:Print**) (<Ctrl + P>)

Print all (**File:Print All**) (<Ctrl + Shift + P>)

Report (**File:Report**)

Report alignment (**File:Report Alignment**)
Export to metafile (**File:Export to Metafile**)
Export (**File:Export**)
Export alignment (**File:Export Alignment**)
Split file (**File:Split File**)
Copy (**Edit:Copy Clone/Sequence/Gel**) (<Ctrl + C>)

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